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STUDIES IN HUMAN INHERITANCE XIII

A TABLE TO DETERMINE THE EXPECTED PROPORTION OF FEMALES SHOWING A SEX-INFLUENCED CHARACTER CORRESPONDING TO ANY GIVEN PROPORTION OF MALES SHOWING THE CHARACTER

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Received September 1, 1935

IN A PREVIOUS paper (Snyder, L. H. and Yingling, H., 1935) the gene frequency method was applied to sex-influenced factors, and a formula for testing the applicability of the hypothesis of sex-influenced factors to human data was derived. Since any character dependent upon sex-influenced factors will usually be relatively frequent in males, but relatively rare in females, another method of attack presents itself. Knowing the proportion of males showing a character suspected of being due to a sex-influenced factor, what proportion of females may be expected to show the character, assuming random mating? As a practical example, if 40% of males are bald, what proportion of females may be expected to be bald, assuming that baldness is due to a sex-influenced factor and that random mating occurs in regard to this character?

Assume a pair of allelomorphs B and b, such that B is dominant in males, but recessive in females. Let p = frequency of B, and q = frequency of b. Then p + q = 1. Here p and q may be separately derived, as follows:

$$\frac{p^2}{2} = \text{proportion of } BB\,♀\,♀ \text{ in general population,}$$

$$\frac{q^2}{2} = \text{proportion of } bb\,♂\,♂ \text{ in general population.}$$

Since the proportion of $BB\,♀\,♀$ in the general population is equal to half of their proportion among females alone, and the proportion of $bb\,♂\,♂$ in the general population is equal to half of their proportion among males alone, we may write as follows:

Let $\overline{♀\,B}$ = proportion among females of females who show the character represented by B, let $\overline{♂B}$ = proportion among males of males who show the character represented by B, and let $\overline{♂b}$ = proportion among males of males who show the character represented by b. Then

$$\frac{p^2}{2} = \frac{\overline{♀\,B}}{2}$$

$$p = \sqrt{\overline{♀\,B}} \qquad (1)$$

$$\frac{q^2}{2} = \frac{\overline{♂b}}{2}$$

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$$q=\sqrt{\overline{\sigma b}}. \tag{2}$$

So that

$$\sqrt{\overline{\varphi B}}+\sqrt{\overline{\sigma b}}=1. \tag{3}$$

From equation (3) it is possible to derive a value of $\overline{\varphi B}$ in terms of $\overline{\sigma B}$.

$$\begin{aligned}\sqrt{\overline{\varphi B}}+\sqrt{\overline{\sigma b}}&=1\\ \sqrt{\overline{\varphi B}}&=1-\sqrt{\overline{\sigma b}}=1-\sqrt{1-\overline{\sigma B}}\\ \overline{\varphi B}&=\left(1-\sqrt{1-\overline{\sigma B}}\right)^2.\end{aligned} \tag{4}$$

From equation (4) a table may be constructed, giving, for any proportion of males showing a dominant sex-influenced character, the corresponding proportion of females who may be expected to show the character. Employing the maximum likelihood method of R. A. FISHER (1930), the probable error formula for equation (4) may be derived as follows:

The distribution of dominant and recessive individuals in a sample of N males follows the terms of the expansion of the binomial, $[(p^2+2pq)+q^2]^N$. More exactly, the chance P of getting n dominants and N−n recessives is

$$P=K(p^2+2pq)^n(q^2)^{N-n}=K(1-q^2)^n(q^2)^{N-n} \tag{5}$$

where

$$K=\frac{N!}{n!(N-n)!}.$$

The value to be estimated is p^2, so that setting

$$\theta=p^2$$

we obtain

$$q=1-\sqrt{\theta}.$$

Putting this value of q in equation (5), we get

$$P=K(2\sqrt{\theta}-\theta)^n(1-\sqrt{\theta})^{2N-2n}. \tag{6}$$

Taking natural logarithms,

$$\begin{aligned}L&=\log P=\log K+n\log(2\sqrt{\theta}-\theta)+(2N-2n)\log(1-\sqrt{\theta})\\ \frac{\partial L}{\partial\theta}&=\frac{n(1-\sqrt{\theta})}{\theta(2-\sqrt{\theta})}-\frac{N-n}{\sqrt{\theta}-\theta}\\ \frac{\partial^2 L}{\partial\theta^2}&=\frac{(N-n)(1-2\sqrt{\theta})}{2\sqrt{\theta}(\sqrt{\theta}-\theta)^2}-\frac{n}{2\theta\sqrt{\theta}(2-\sqrt{\theta})^2}-\frac{n(1-\sqrt{\theta})}{\theta^2(2-\sqrt{\theta})}.\end{aligned} \tag{7}$$

Substituting $\theta=p^2$ and $n=Np(2-p)$, we have

$$\frac{\partial^2 L}{\partial(p^2)^2}=-\frac{N}{p^3(2-p)}. \tag{8}$$

The variance of p^2,

$$\sigma^2 p^2 = -\frac{1}{\frac{\partial^2 L}{\partial (p^2)^2}} = \frac{p^3(2-p)}{N} . \tag{9}$$

Substituting $p = 1 - \sqrt{1 - \overline{\sigma^7 B}}$, we get

$$\text{P.E. } p^2 = \pm .6745\left(1 - \sqrt{1 - \overline{\sigma^7 B}}\right)\sqrt{\frac{\overline{\sigma^7 B}}{N}} . \tag{10}$$

The complete equation involved is thus

$$\overline{♀ B} = \left(1 - \sqrt{1 - \overline{\sigma^7 B}}\right)^2 \pm .6745\left(1 - \sqrt{1 - \overline{\sigma^7 B}}\right)\sqrt{\frac{\overline{\sigma^7 B}}{N}} . \tag{11}$$

From table 1, for any proportion of males showing a dominant sex-influenced character, the proportion of females who may be expected to show the character may be directly read.

TABLE 1

Table of values of $\overline{♀ B} = \left(1 - \sqrt{1 - \overline{\sigma^7 B}}\right)^2$

The values of $\overline{\sigma^7 B}$ *to two decimal places are given in the left-hand column; the third decimal place for each value is given in the top row. Thus for* $\overline{\sigma^7 B} = .310$, *the proportion of* $\overline{♀ B}$ *is .0287; for* $\overline{\sigma^7 B} = .316$ *it is .0299.*

$\overline{\sigma^7 B}$	.000	.001	.002	.003	.004	.005	.006	.007	.008	.009
.000	.0000	.0000	.0000	.0000	.0000	.0000	.0000	.0000	.0000	.0000
.010	.0000	.0000	.0000	.0000	.0000	.0001	.0001	.0001	.0001	.0001
.020	.0001	.0001	.0001	.0001	.0001	.0002	.0002	.0002	.0002	.0002
.030	.0002	.0002	.0003	.0003	.0003	.0003	.0003	.0003	.0004	.0004
.040	.0004	.0004	.0005	.0005	.0005	.0005	.0005	.0006	.0006	.0006
.050	.0006	.0007	.0007	.0007	.0007	.0008	.0008	.0008	.0009	.0009
.060	.0009	.0010	.0010	.0010	.0011	.0011	.0011	.0012	.0012	.0012
.070	.0013	.0013	.0013	.0014	.0014	.0015	.0015	.0015	.0016	.0016
.080	.0017	.0017	.0018	.0018	.0018	.0019	.0019	.0020	.0020	.0021
.090	.0021	.0022	.0022	.0023	.0023	.0024	.0024	.0025	.0025	.0026
.100	.0026	.0027	.0027	.0028	.0029	.0029	.0030	.0030	.0031	.0031
.110	.0032	.0033	.0033	.0034	.0034	.0035	.0036	.0036	.0037	.0038
.120	.0038	.0039	.0040	.0040	.0041	.0042	.0042	.0043	.0044	.0045
.130	.0045	.0046	.0047	.0047	.0048	.0049	.0050	.0050	.0051	.0052
.140	.0053	.0054	.0054	.0055	.0056	.0057	.0058	.0058	.0059	.0060
.150	.0061	.0062	.0063	.0063	.0064	.0065	.0066	.0067	.0068	.0069
.160	.0070	.0071	.0072	.0072	.0073	.0074	.0075	.0076	.0077	.0078
.170	.0079	.0080	.0081	.0082	.0083	.0084	.0085	.0086	.0087	.0088
.180	.0089	.0090	.0091	.0092	.0093	.0095	.0096	.0097	.0098	.0099
.190	.0100	.0101	.0102	.0103	.0104	.0106	.0107	.0108	.0109	.0110

TABLE 1. (*Continued*)

Table of Values of $\overline{♀}B=\left(1-\sqrt{1-\overline{♂}B}\right)^2$

$\overline{♂}B$	.000	.001	.002	.003	.004	.005	.006	.007	.008	.009
.200	.0111	.0113	.0114	.0115	.0116	.0117	.0119	.0120	.0121	.0122
.210	.0124	.0125	.0126	.0127	.0129	.0130	.0131	.0133	.0134	.0135
.220	.0136	.0138	.0139	.0140	.0142	.0143	.0145	.0146	.0147	.0149
.230	.0150	.0151	.0153	.0154	.0156	.0157	.0159	.0160	.0161	.0163
.240	.0164	.0166	.0167	.0169	.0170	.0172	.0173	.0175	.0176	.0178
.250	.0179	.0181	.0183	.0184	.0186	.0187	.0189	.0191	.0192	.0194
.260	.0195	.0197	.0199	.0200	.0202	.0204	.0205	.0207	.0209	.0210
.270	.0212	.0214	.0215	.0217	.0219	.0221	.0222	.0224	.0226	.0228
.280	.0229	.0231	.0233	.0235	.0237	.0238	.0240	.0242	.0244	.0246
.290	.0248	.0250	.0251	.0253	.0255	.0257	.0259	.0261	.0263	.0265
.300	.0267	.0269	.0271	.0273	.0275	.0277	.0279	.0281	.0283	.0285
.310	.0287	.0289	.0291	.0293	.0295	.0297	.0299	.0301	.0303	.0305
.320	.0308	.0310	.0312	.0314	.0316	.0318	.0320	.0323	.0325	.0327
.330	.0329	.0332	.0334	.0336	.0338	.0340	.0343	.0345	.0347	.0350
.340	.0352	.0354	.0357	.0359	.0361	.0364	.0366	.0368	.0371	.0373
.350	.0375	.0378	.0380	.0383	.0385	.0388	.0390	.0393	.0395	.0398
.360	.0400	.0403	.0405	.0408	.0410	.0413	.0415	.0418	.0420	.0423
.370	.0425	.0428	.0431	.0433	.0436	.0439	.0441	.0444	.0447	.0449
.380	.0452	.0455	.0457	.0460	.0463	.0466	.0468	.0471	.0474	.0476
.390	.0479	.0482	.0485	.0488	.0491	.0494	.0497	.0499	.0502	.0505
.400	.0508	.0511	.0514	.0517	.0520	.0523	.0526	.0529	.0532	.0535
.410	.0538	.0541	.0544	.0547	.0550	.0553	.0556	.0559	.0562	.0565
.420	.0568	.0572	.0575	.0578	.0581	.0584	.0587	.0591	.0594	.0597
.430	.0600	.0604	.0607	.0610	.0613	.0617	.0620	.0623	.0627	.0630
.440	.0633	.0637	.0640	.0644	.0647	.0650	.0654	.0657	.0661	.0664
.450	.0668	.0671	.0675	.0678	.0682	.0685	.0689	.0692	.0696	.0699
.460	.0703	.0707	.0710	.0714	.0718	.0721	.0725	.0729	.0732	.0736
.470	.0740	.0744	.0747	.0751	.0755	.0758	.0762	.0766	.0770	.0774
.480	.0778	.0782	.0786	.0789	.0793	.0797	.0801	.0805	.0809	.0813
.490	.0817	.0821	.0825	.0829	.0832	.0837	.0841	.0846	.0850	.0854
.500	.0858	.0862	.0866	.0870	.0875	.0879	.0883	.0887	.0891	.0896
.510	.0900	.0904	.0909	.0913	.0917	.0922	.0926	.0930	.0935	.0939
.520	.0944	.0948	.0952	.0957	.0961	.0966	.0970	.0975	.0980	.0984
.530	.0989	.0993	.0998	.1003	.1007	.1012	.1016	.1021	.1026	.1031
.540	.1035	.1040	.1045	.1050	.1054	.1059	.1064	.1069	.1074	.1079
.550	.1084	.1089	.1093	.1098	.1103	.1108	.1113	.1118	.1123	.1128
.560	.1134	.1139	.1144	.1149	.1154	.1159	.1164	.1169	.1175	.1180
.570	.1185	.1190	.1196	.1201	.1206	.1212	.1217	.1222	.1228	.1233
.580	.1239	.1244	.1249	.1255	.1260	.1266	.1271	.1277	.1283	.1288
.590	.1294	.1299	.1305	.1311	.1316	.1322	.1328	.1334	.1339	.1345

TABLE 1. (*Continued*)

Table of Values of $\overline{♀B}=\left(1-\sqrt{1-\overline{♂B}}\right)^2$

$\overline{♂B}$	.000	.001	.002	.003	.004	.005	.006	.007	.008	.009
.600	.1351	.1357	.1363	.1368	.1374	.1380	.1386	.1392	.1398	.1404
.610	.1410	.1416	.1422	.1428	.1434	.1440	.1446	.1453	.1459	.1465
.620	.1471	.1477	.1484	.1490	.1496	.1502	.1509	.1515	.1521	.1528
.630	.1534	.1541	.1547	.1554	.1560	.1567	.1574	.1580	.1587	.1593
.640	.1600	.1607	.1613	.1620	.1627	.1634	.1640	.1647	.1654	.1661
.650	.1668	.1675	.1682	.1689	.1696	.1703	.1710	.1717	.1724	.1731
.660	.1738	.1745	.1752	.1760	.1767	.1774	.1781	.1789	.1796	.1803
.670	.1811	.1818	.1826	.1833	.1841	.1848	.1856	.1863	.1871	.1879
.680	.1886	.1894	.1902	.1909	.1917	.1925	.1933	.1941	.1949	.1957
.690	.1964	.1972	.1980	.1988	.1997	.2005	.2013	.2021	.2029	.2037
.700	.2046	.2054	.2062	.2070	.2079	.2087	.2096	.2104	.2113	.2121
.710	.2130	.2138	.2147	.2156	.2164	.2173	.2182	.2190	.2199	.2208
.720	.2217	.2226	.2235	.2244	.2253	.2262	.2271	.2280	.2289	.2298
.730	.2308	.2317	.2326	.2336	.2345	.2354	.2364	.2373	.2383	.2392
.740	.2402	.2412	.2421	.2431	.2441	.2450	.2460	.2470	.2480	.2490
.750	.2500	.2510	.2520	.2530	.2540	.2551	.2561	.2571	.2581	.2592
.760	.2602	.2612	.2623	.2633	.2644	.2655	.2665	.2676	.2687	.2698
.770	.2708	.2719	.2730	.2741	.2752	.2763	.2774	.2785	.2797	.2808
.780	.2819	.2831	.2842	.2853	.2865	.2876	.2888	.2900	.2911	.2923
.790	.2935	.2947	.2959	.2971	.2983	.2995	.3007	.3019	.3031	.3043
.800	.3056	.3068	.3081	.3093	.3106	.3118	.3131	.3144	.3156	.3169
.810	.3182	.3195	.3208	.3221	.3234	.3248	.3261	.3274	.3288	.3301
.820	.3315	.3328	.3342	.3356	.3370	.3383	.3397	.3411	.3425	.3440
.830	.3454	.3468	.3482	.3497	.3511	.3526	.3540	.3555	.3570	.3585
.840	.3600	.3615	.3630	.3645	.3661	.3676	.3691	.3707	.3723	.3738
.850	.3754	.3770	.3786	.3802	.3818	.3834	.3851	.3867	.3883	.3900
.860	.3917	.3933	.3950	.3967	.3984	.4002	.4019	.4036	.4054	.4071
.870	.4089	.4107	.4125	.4143	.4161	.4179	.4197	.4216	.4234	.4253
.880	.4272	.4291	.4310	.4329	.4348	.4368	.4387	.4407	.4427	.4447
.890	.4467	.4487	.4507	.4528	.4548	.4569	.4590	.4611	.4633	.4654
.900	.4675	.4697	.4719	.4741	.4763	.4786	.4808	.4831	.4854	.4877
.910	.4900	.4923	.4947	.4971	.4995	.5019	.5043	.5068	.5093	.5118
.920	.5143	.5169	.5194	.5220	.5246	.5273	.5299	.5326	.5353	.5381
.930	.5408	.5436	.5465	.5493	.5522	.5551	.5580	.5610	.5640	.5670
.940	.5701	.5732	.5763	.5795	.5827	.5860	.5892	.5926	.5959	.5993
.950	.6028	.6063	.6098	.6134	.6170	.6207	.6245	.6283	.6321	.6360
.960	.6400	.6440	.6481	.6523	.6565	.6608	.6652	.6697	.6742	.6789
.970	.6836	.6884	.6933	.6984	.7035	.7088	.7142	.7197	.7254	.7312
.980	.7372	.7433	.7497	.7562	.7630	.7701	.7774	.7850	.7929	.8012
.990	.8100	.8193	.8291	.8397	.8511	.8636	.8775	.8935	.9126	.9378

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THE TEMPERATURE-EFFECTIVE PERIODS AND THE GROWTH CURVES FOR LENGTH AND AREA OF THE VESTIGIAL WINGS OF DROSOPHILA MELANOGASTER

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INTRODUCTION

ROBERTS (1918) first reported the increase in size of the wings of the vestigial mutant of *D. melanogaster* when reared at high temperatures and showed that the effect was more pronounced in the males than in the females. STANLEY (1928) stated that "the length of vestigial wing varies directly with the temperature, but not in direct proportion." I have shown (1930a) that there is a critical temperature for the increase in the length of the vestigial wings and that the marked change occurs in the females at a temperature 1° higher than in the males, a response showing sexual dimorphism. The curves for total development at one temperature of STANLEY (1931) and mine (1930a) were similar over the temperature range examined (17° to 31°). In preliminary reports (1930b, 1932, 1933) I have indicated that the temperature-effective period for increasing the size of the vestigial wings at 30°, 31°, 32°, and 33° C. is during the larval period commencing at approximately 60 hours and extending for various intervals with different maximum rates depending upon the sex of the individuals and the temperature experienced by the larvae. STANLEY (1931) found entirely different temperature-effective periods at 17° and 27° but in his recent paper (1935) he reports intervals at 30° and 31° easily harmonized with the periods reported here.

STOCKS AND METHODS

An inbred stock of vestigial winged flies should be homozygous for practically all the genes concerned in wing development and consequently the variability due to recombinations of modifying genes would be reduced to a minimum. Single pair brother-sister matings were made and selection was practiced in each generation, the sib matings being made from the bottle showing the best yield and with the flies showing the largest vestigial wings. The selection practiced should accelerate the approach to homozygosity.

The food used in the experiments was the customary banana-agar jell. The bananas used throughout the experiments were uniformly overripe (the skins dark brown), but not decayed, and a 1 percent agar-agar me-

dium was used. The food was poured to a depth of 25 mm. in 1×4 inch vials and yeasted 24 hours before the parents entered the vials. The same batch of food was used at both temperatures and for all time intervals in any one trial.

In the final experiments the same parents produced the eggs for the trials at 30° and 32°, 31° and 32°, and 31° and 33° C.; repeated trials being alternated between these temperature combinations. Thus comparable data should be produced for these four temperatures. One-hour egg-laying periods at 25° with eight pairs of flies per vial were used for the experiments at 30°, 31°, and 32°. The number of eggs deposited in each vial was below the limits of "the crowding effect" on the wings. These vials remained in the 25° incubator for 24 hours. At the end of this period practically all the eggs had hatched HARNLY (1929). Preliminary tests had shown no temperature effect on the wings during the first 48 hours of development. The vials were then placed at two of the experimental temperatures. Eggs from the same parents were placed at all time intervals at both temperatures, thus distributing any residual genetic variability uniformly throughout the experiment. Beginning at 48 hours of total development and every 4 hours thereafter sets of vials were returned to the 25° incubators for the completion of development and the emergence of the adult flies.

A temperature of 33° was nearly 2° above the lethal point for the complete development and emergence of the flies of this vestigial stock. A procedure was developed which would carry a fair number to puparium formation at 33°. A 3-hour egg-laying interval was used and the first 48 hours of development took place at 25°. The larvae in vials transferred at this time from 25° to 33° entered the temperature-effective period some twelve or more hours later. Beginning at 72 hours of total development and every 12 hours thereafter sets of vials were transferred from 33° to 25° for the completion of development and emergence of the adult flies. The larvae had great difficulty in forming puparia at 33°. Most of them moved up on the walls of the vials and crawled around on the surface of the glass for several hours until death resulted apparently from excessive drying. To overcome this hazard the larvae were removed from the walls of the vials with a moistened camel's hair brush every 2 to 4 hours and returned to the surface of the food during the puparium formation period at 33°. With this procedure many of them succeeded in forming puparia and, if placed at a lower temperature presumably before the time of pupation proper, succeeded in completing their development and emerging as adult flies. No emerged flies have been obtained from total development at either 32° or 33° with this stock. Total and partial development was tried repeatedly at 34°, 35°, and 36° without much success.

The accuracy of the incubators used was ± 0.05° C., the temperatures being determined through the double glass door with burette readers from standardized thermometers graduated to 0.1° C. The incubators used in our laboratory for temperature work are all small units with only one shelf and an electric fan maintains a forced circulation of the air within the incubator. The thermostats are grids of glass tubing containing toluol and are closed by a mercury column having a movement in the capillary arm of an inch and a half per degree centigrade. The thermostat carries a very small current thus preventing sparking and fouling of the mercury surface and it controls the polarity on the grid of a radio tube. This in turn operates a telegraph relay carrying on its rocker arm a tube in which the heating circuit is made and broken in mercury, thus removing the common difficulty of frozen contact points in the heating circuit and the resultant death of the cultures. The lag is negligible and the heating circuit is made and broken on an average of once in 60 to 90 seconds. The air accuracy of these incubators is ±0.05° C. but tests with thermocouples placed in the surface of the food in which the larvae are feeding show no perceptible fluctuation between the on and off position in the temperature of the food surrounding the larvae. This control mechanism will maintain a constant temperature for months without any adjustment. The 25° incubators were kept in a cold room (10° C.) which had a variation of less than 1° and the 30°, 31°, 32°, and 33° incubators were kept in a room where the temperature varied from 20° to 22° C.

The right wings of the emerged flies were removed under a binocular microscope, mounted in 95 percent alcohol under a cover slip, projected with a compound microscope and a 500 watt lamp, and drawn. The linear magnification was 115. The lengths were determined by projecting a Leitz 2 mm slide ruled to 0.01 mm onto the drawings. The areas were determined with a Keuffel and Esser compensating polar planimeter no. 4242. The mean values for each time interval were determined from the lengths and areas of an average of fifty males and fifty females except the last few points at each temperature. Due to the high death rate for long exposures these points are the mean value of twenty-five to forty surviving individuals. Six trials were made at each temperature.

TEMPERATURE-EFFECTIVE PERIODS

A. Preliminary tests

The preliminary tests on the temperature-effective period were reported in London (HARNLY 1930 b). They were made on the twelfth generation of brother-sister matings with transfers from 31° and 32° to 29° and reciprocal transfers from 29° to 31° C. Since the preliminary experiments had shown that the temperature-effective period began late in the third day

of development it was not necessary in the final tests to make transfers from the high temperatures to 25° before the first 48 hours of development had been completed. This allowed a check interval at the high temperatures of over 12 hours before the beginning of the critical period.

B. Periods and growth curves for length and area

The final experiments were performed at 30°, 31°, and 32° with transfers at 4-hour intervals to 25° C. and at 33° with 12-hour transfer periods. These experiments extended from the thirty-first through the fiftieth generation of the sib matings. In figures 1, 2, and 3 are plotted the mean length

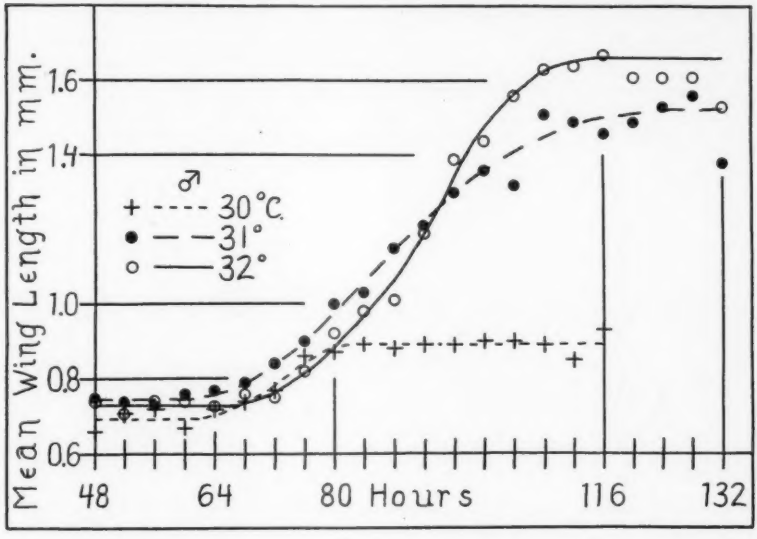

FIGURE 1

of the male and female vestigial wings for the total developmental time intervals at which the individuals were transferred from the higher temperature to the 25° incubators for the completion of development and emergence.

From the curves (smoothed by eye) it is evident that the temperature-effective period for wing length began for the males at 30° between 60 and 64 hours and ended between 80 and 84 hours of total development, a duration of some 20 hours, and terminating 27 hours before the mean time of puparium formation (111 hours). At 31° the rise began in the curve for the males at the same time as at 30° (that is, 64 hours) but instead of leveling off at 84 hours it continued upward as a sigmoid curve approaching its asymtote at 112 hours, the mean time of puparium formation for this

stock at 31°. A rise of 1° had affected neither the length of the egg-larval period nor the inception of the temperature-effective period but had changed the termination time; its duration being prolonged from 20 hours to 48 hours, an increase of 140 percent, accompanied by a 67 percent increase in wing length. Thirty-one degrees was close to the lethal point and 32° was above it for total development. Furthermore the larval period was prolonged something over 24 hours at 32° and the initiation of the temperature-effective period was retarded approximately 4 hours (68 hours) in comparison with 30° and 31° (64 hours). The duration of the effective period was again 48 hours (to 116 hours), though puparium for-

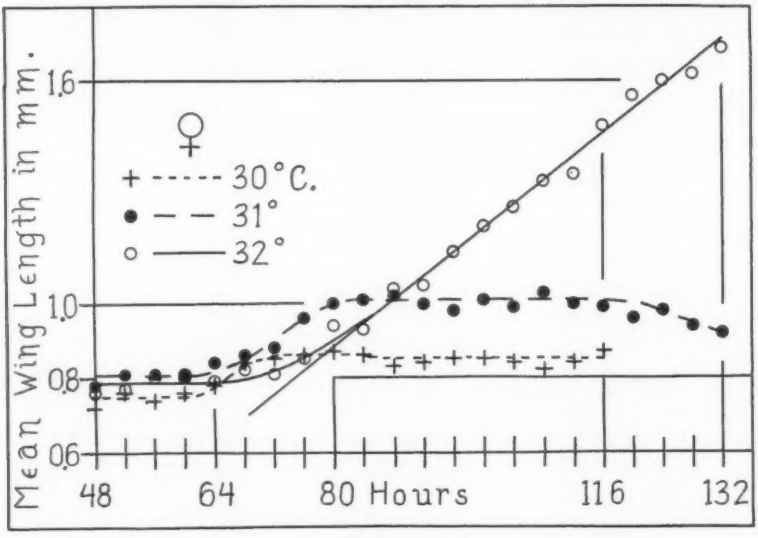

FIGURE 2

mation did not come until much later. The wings of the males at 32° were longer than those developed at 31°; this increase of 1° had not affected the duration of the temperature-effective period but did result in a higher maximum rate of wing development. The major change in the vestigial wings of the males at 32° was in the area. Due to the high mortality during the latter part of the developmental period at 33° only sufficient data was obtained to establish the five points between 72 hours and 120 hours. The linear equation

$$y = 0.01592x - 0.357$$

appears to describe these five points.

The female period for wing length at 30° began between 60 and 64 hours and ended between 68 and 72 hours of total development (puparium

formation 107 hours), an effective period of some 8 hours. Commencing at the same time at 31° the effective period was lengthened from 8 to 20 hours, the curve leveling off between 80 and 84 hours. The termination point for the females at 31° was not associated with puparium formation which came some 30 hours later (114 hours). The growth curve for the females at 32° started approximately 4 hours later than at 31° but instead of leveling off around 84 hours it proceeded upward apparently as a straight line through the rest of the time interval examined. The linear equation

$$y = 0.01592x - 0.391$$

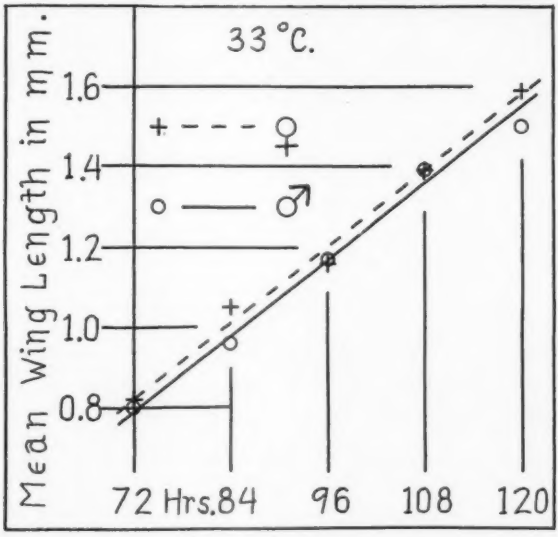

FIGURE 3

appears to describe all the points determined during this temperature-effective period with the exception of the first few points. It is obvious from the data and an inspection of the figures that the rises of 1° from 30° to 31° and from 31° to 32° though they did not significantly affect the inception of the temperature-effective period in the females did markedly affect its termination, the periods at 30°, 31°, and 32° being respectively 8, 20 and some undetermined period more than 70 hours in duration. The five points at 33° for which sufficient data was obtained appear to fit the equation

$$y = 0.01592x - 0.326.$$

The equations for the females at 32° and the males and females at 33° differ only in the intercept, the slope being the same for all three.

The close fit of the straight line drawn for the females at 32° to all the points determined experimentally, except the first few points in the temperature-effective period, raises the question as to whether or not the beginning of this period was sharply defined in time. From the curves in figures 1 and 2, either the temperature-effective period began at the same moment in time for all the individuals and the rate increased during the first few hours, or individuals all of the same chronological age entered this critical period of ontogeny at different times and each of the first two or three points represented more and more individuals in the effective period. Even the most superficial examination of the drawings for either

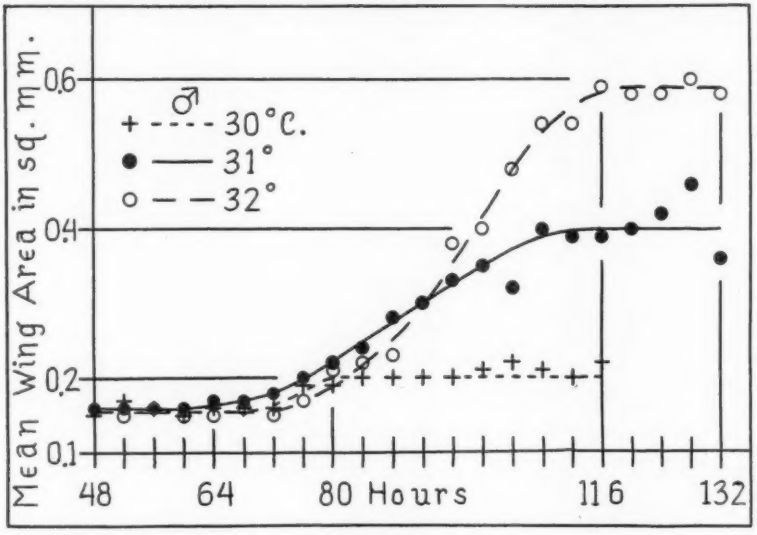

FIGURE 4

sex at any one of these temperatures demonstrates conclusively that the latter alternative was correct for both the beginning and end of the period. The temperature-effective period as used here denotes that interval in time from the entrance of the first few larvae into the critical period until the last have passed beyond it. The value is that of the whole population. In work of this type with *D. melanogaster* it is impossible to determine this value for a single individual since only one measurement in time can be made on it. The results presented here make possible a new technique by which more accurate values will probably be obtained in subsequent work with other alleles at this locus. Attention has been called to this problem in recent work on the scute and Bar loci (CHILD 1935, MARGOLIS 1935).

The wing areas are plotted against time of transfer in figures 4, 5, and 6. When one considers the data for the males at 30°, 31°, and 32° in figures 1 and 4 it is obvious that there are only minor differences in the curves and time intervals for the length and for the area of the wings. The effective period for wing area began slightly later than that for wing length. It would seem that the beginning of the period involved a slight increase in length at the expense of the width with no resultant change in area. The termination times for both length and area seem to have coincided at each temperature. For area as for length the duration of the temperature-effective period was prolonged markedly with a rise of 1° from 30°

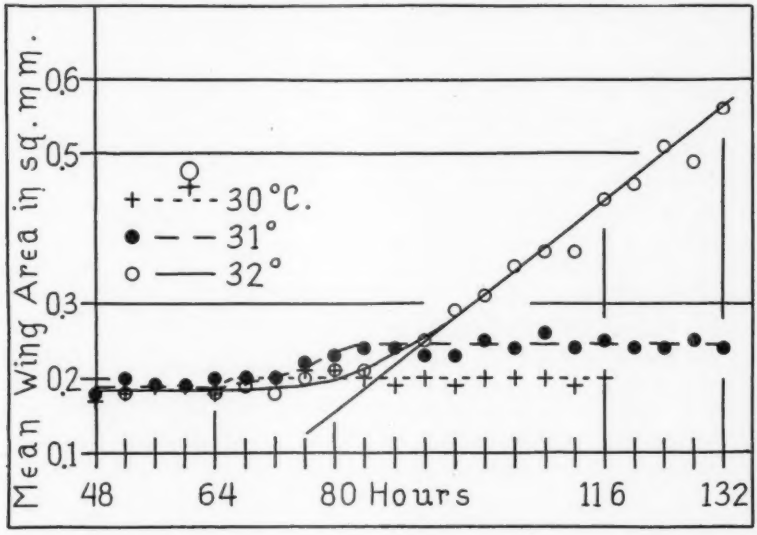

FIGURE 5

to 31° and there was a pronounced increase in the maximum rate with no significant change in the duration at 32°. The major change in wing form from 31° to 32° was in the width and area of the wing accompanied by only a minor increase in wing length. The curve for area at 33° was very definitely not linear and the wing forms were similar to those at 32° for the males.

The changes in wing length and wing area for the females were likewise very similar. The beginning of the temperature-effective period seemed to come a little later for wing area than for wing length but they both ended apparently at the same time for each temperature. The rises of 1° from 30° to 31° and from 31° to 32° each lengthened the effective period significantly. The five points determined for wing area of the females at 33° were not linear.

The sexual dimorphism previously reported at high temperatures (ROBERTS 1918; HARNLY 1930a, b; STANLEY 1928, 1931) was obviously due to differences between the males and the females both in rates of "wing formation" and durations of the effective period at each temperature. At 30° the length of the temperature-effective period of the males was several times that of the females. The effective period for the females at 31° was 20 hours (male duration at 30°) and the curve was practically identical in time with that for the males at 30°, but with a higher value throughout. However, the male duration had changed at 31° from 20 hours to 48 hours, continuing the sexual dimorphism at this temperature. A rise of 1° did not modify the inception of the temperature-effective period but had retarded its termination differently for the two sexes. The same length and area was attained in the wings of the males and the females at 32° but it is very evident from the curves that the growth processes were markedly different and the effective period was probably considerably longer for the females than for the males. If they could have been carried for a longer period at 32° the curves indicate that the wings of the females might have been larger than those of the males at this temperature. In the trials at 32° sets of vials were carried for transfer periods considerably beyond 132 hours since it was known that puparium formation came later there. Unfortunately 132 hours was practically the time toleration limit and only a few individuals were obtained beyond that point. These occasional survivors had still larger wings and, though they do not prove it, agree with the assumption that if development could go through to completion of the temperature-effective period for the females at 32°, the two sexes would still show a difference in wing size, but at this point the wings of the females would be larger than those of the males; a complete reversal of the sexual dimorphism to that found in the wild type wings.

In the light of this assumption the curves for the males and the females at 33° are of interest. The mean values for the wings of the females were larger than those of the males at the five points examined between 72 and 120 hours (figures 3, 6), a reversal of the position of the growth curves of the two sexes for vestigial. It has been shown that the wild type wing and many other structures vary inversely with temperature (LUTZ 1913, ALPATOV and PEARL 1929, ALPATOV 1930b, EIGENBRODT 1930, IMAI 1933, STANLEY 1935). STANLEY (1935) has found that the wings of homozygous long-winged (wild type) females were consistently and markedly longer than those of the males for each transfer interval during the temperature-effective periods at 17°, 27°, and 30° C. and the longer the developmental period passed at the higher temperatures, the smaller the wings. Both variation with temperature and with time and the relative sizes of the

wings of the two sexes were reversed in homozygous vestigial flies as opposed to the normal allele. But at 33°, though the wing size still increased with time its relative size for the males and females had been changed toward the wild type condition. This indicates that there had been a change in the developmental processes producing in these curves of the male and female vestigial genotypes at 33° a partial simulation of the normal long-wing genotype growth curves. However it was only a partial simulation since wing size for the vestigial flies still varied directly with the length of time the larvae were exposed to 33°. Furthermore there was never any close approach to the normal phenotype in either sex genotypi-

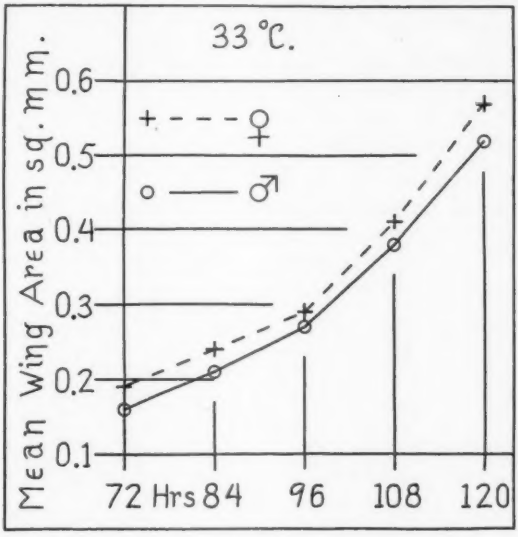

FIGURE 6

cally homozygous for vestigial. As I have pointed out before (HARNLY 1932), the mutant gene vestigial seems to determine the minimum and maximum size of the wings, the potential size being determined by the vestigial gene together with other genes affecting the wings and the exact expression is determined by the gene complex acting in a specific environment during development.

From the data presented above it was evident that the temperature-effective period ended long before the time of puparium formation for the males and the females at 30° and for the females at 31° though here the two events seemed to coincide in the males. At 32° the effective period terminated in the males long before puparium formation but this relationship could not be determined for the females due to the lethal effect of

exposures beyond 132 hours at this temperature. An experiment was performed to determine whether or not the effective period had ended in the males by the time puparia were formed at 31°.

C. Termination test

Eggs from the same parents were obtained by repeated 1-hour egg-laying periods and allowed to hatch at 25° (24 hours development). The vials were then transferred to 31° for the larval period. With the beginning of puparium formation hourly examinations were made and the newly formed puparia isolated on agar-agar slants in vials. Each hourly set of puparia (prepupae) from a given vial were divided into three groups, consequently any differences due to the number of hours passed in the egg-larval period would be uniformly distributed through the test. At each hourly isolation the first group of puparia were immediately placed at 25° for further development and emergence; the second group of isolated puparia passed an additional 6 hours at 31° and were then transferred to the 25° incubators; and the third group spent 12 hours more at 31° (by which time pupation proper had occurred in all of them) before they were placed in the 25° incubator for further development and emergence. There was no further increase in size due to exposure to 31° beyond the hour of puparium formation.

TEMPERATURE AND GENERAL RATE OF DEVELOPMENT

Any interpretation of the results depends on whether these high temperatures affected proportionately or disproportionately the duration of the effective period and the duration of the general developmental periods, especially the duration of the egg-larval period. The following experiments were performed to answer this question.

A. Methods

The usual procedure for the exact timing of development was followed (Li 1927, Harnly 1929). Single pair matings of the inbred vestigial flies were used. The eggs were deposited on blotting-paper slips which were then placed on end in 1×4 inch vials containing food 25 mm deep. All the eggs were laid during 1-hour periods at 25° C. At the end of this interval the slips were removed and so distributed that approximately equal numbers of eggs from each female were placed at 25°, 29°, 30°, 31°, and 32°. All subsequent development took place at the temperatures indicated. Hourly examinations were made during the hatching period and at the time of puparium formation. Each puparium was placed on a 2 percent agar-agar slant in a separate vial. The time of emergence and sex of each adult was determined by hourly examinations of these isolated puparia during the emergence period.

B. Effects on the rate of development

The mean duration of the embryonic period (hours required to hatch the eggs) was 21.7 hours at 25°. This was practically the same value found previously for vestigial flies and the frequency curve conformed to the earlier one showing no signs of a bimodal distribution (HARNLY 1929). The frequency distribution curves for hatching at the higher temperatures were also simple sharp unimodal curves. The maximum effect occurred between 25° and 29°, and over the range of 29° to 32° there was no significant change in the duration of this developmental period (19.4 to 19 hours). The major effect of temperature on the length of the embryonic period occurred below the critical temperatures which were found to increase the size of the vestigial wings (30° ♂♂, 31° ♀♀).

The durations of the egg-larval periods (hours from egg-laying to puparium formation) for the males and the females are stated in table 1. An increase of 4° (25° to 29°) reduced the egg-larval period 17 hours (14 percent) for the males and 10 hours (8.6 percent) for the females, these changes appearing in a temperature range below the critical points for the wings of the males and the females. A rise from 29° through the critical point for the vestigial wings of the males to 30° produced an increase of 6.7 hours in the egg-larval period of the males, but no change occurred in the duration of the egg-larval period for the females. Conversely a rise in temperature through the critical point for the wings of the females from 30° to 31° had only a very slight effect on the egg-larval period of the males but lengthened this period 7.2 hours for the females. The lethal point for complete development of the vestigial winged flies was practically reached at 31°, very few adults emerging, and had been passed at 32°. Consequently the egg-larval period was determined for those larvae which formed puparia at 32° and as no adult flies emerged the sexes were not separated.

TABLE 1

Duration of developmental periods in hours, 4 trials.

°C.	EGG-LARVAL		EGG-LARVAL-PUPAL	
	MEAN ± P.E.	σ	MEAN ± P.E.	σ
25♂	121.35 ± 1.37	10.37	229.81 ± 1.48	11.18
♀	117.37 ± 1.09	8.38	219.42 ± 1.39	10.50
29♂	104.14 ± 0.82	6.53	192.69 ± 0.91	7.26
♀	106.78 ± 0.87	8.26	192.63 ± 0.96	9.13
30♂	110.86 ± 1.61	12.83	195.79 ± 1.10	8.75
♀	106.83 ± 0.82	5.95	189.88 ± 0.83	6.02
31♂	112.22 ± 1.03	4.59	199.78 ± 1.42	6.32
♀	114.00 ± 0.97	3.22	197.80 ± 1.63	5.42
32	140 07 ± 1.64	18.55		

From the egg-larval-pupal periods and the differences between them and the egg-larval periods in table 1 there was evidently little change in this period above 29° through the critical temperatures for the males and the females (30° and 31°). The pupal period may be ignored in this discussion since there was no differential effect of temperature through the critical range, and since it has been shown above that the temperature-effective period fell entirely within the larval period of development.

The difference of 6.7 hours in the egg-larval periods between the males at 29° and 30° represented an increase of 6.4 percent in this period and was 3.72 times the probable error which is just statistically significant and is based on only a very limited number of surviving individuals. In comparison with this small and questionable increase of the egg-larval period from 29° to 30° a pronounced lengthening of the wings by some 35 percent occurred with the same rise of 1° in temperature (HARNLY 1930a). Furthermore at 30° the temperature-effective period was 20 hours and bore no obvious relation to either the small questionable increase of the egg-larval period or to the periods found by STANLEY (1931) at 17° and 27°. As has been shown above with identical egg-larval periods at 30° and 31° there was an increase of 140 percent in the duration of the temperature-effective period and the wings were lengthened 67 percent at 31°. The same situation was found throughout for the females. In the light of these facts and all the data it seems probable that the effects of temperature above 29° on the duration of the major periods of development (embryonic, egg-larval, and egg-larval-pupal) offer no explanation of either the critical temperatures or the sexual dimorphism. Temperature through its effect on the duration of the critical period for wing development and the rate during that period affects not only the length and area of the wings produced, but also through the same processes the wing form or pattern. The result is not simply more wing of the same kind but a definite ordered progression toward the normal type of wing.

GROWTH PATTERN OF WING FORM

Genotypically homozygous vestigial flies show an interesting succession of phenotypes when reared at 29°, 30°, 31°, and 32°. Typical vestigial wings are produced at 29° and all temperatures below this point. The wings of the females reared at 30° are still typical but those of their brothers are much larger and in appearance markedly over-sized vestigial wings. The wings of the females at 31° have increased greatly in size as the male wings did at 30° and in form are the same type of over-sized or giant vestigial wing. But the wings typical for their brothers at 31° are no longer vestigial but the equivalents of the mutants strap to antlered in phenotype. Finally at 32° these genotypically vestigial males and

females have wings that are phenotypically the equivalent of exaggerated notched, or the heterozygote Wild/Carved mutants with parts of the lateral margins missing in many cases. The changes of this genotype with temperature simulating phenotypically other alleles of vestigial reared at 25°, together with the complete progression of the genotype dimorphos vestigial through the vestigial allelic series from the phenotype vestigial at 16° to strap, antlered, Snipped, notched, Carved, vestigial-Beaded, nick, nicked, and finally wild or normal at 32°, and the fact that the homozygous genotype dimorphos vestigial through the temperature range of 16° to 32° duplicated all of the phenotypes produced in MOHR's (1932) combinations of mutants at the vestigial locus at 25° C., led to the propounding of a theory of wing pattern in development in time and the

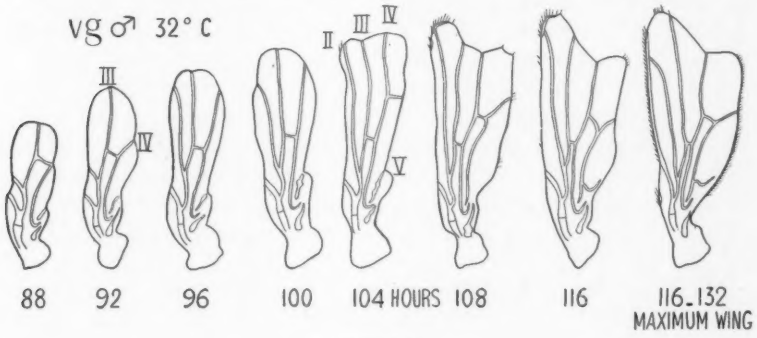

FIGURE 7

hypothesis that mutations at the vestigial locus involved changes in the rate and duration in time of wing development (HARNLY and HARNLY 1935). It must be remembered that mutations at this locus show changes in the form and amount of parts of the wing present, not changes in size only as in some of the other wing loci. The pattern theory advanced involved first growth longitudinally in the region of the II, III, and IV veins with no marked development in width beyond them, followed by growth mainly in the regions of the I and V veins, resulting in a pronounced increase in the width and area of the wing, the last part of the wing developed being the distal margin in the region of the III and IV veins. The same phenotype series has been found for the vestigial genotype in time of development.

This pattern series in ontogeny is shown in figure 7. These wings were the mean types for males spending the number of hours indicated at 32° and completing their development at 25°. The wing at 88 hours was simply an enlarged vestigial wing with a length of 1.01 mm. It was equivalent to

the wings of the homozygous vestigial males reared at 30° (HARNLY 1930a). Growth from then on was mainly longitudinal in the region of the II, III, and IV veins. Twelve hours later (100 hours) the wing was passing through a stage resembling the vestigial-strap mutant, many of the wings being indistinguishable from the published figures of strap wing. Four hours later (104 hours) the wings were similar to the allele antlered wing figured by MOHR (1932). Beyond this interval the bulk of the growth involved the regions of the I and especially the V veins with only minor increases in length. The maximum wing type was obviously a slight exaggeration of the vestigial allele notched-wing. The vestigial genotype during its development at 32° had passed in phenotype successively through the alleles vestigial, strap, antlered, and notched. The sequence of wing patterns for the females at 32° was the same, but had different time values since the rate was much slower in the females (see figures 1 and 2, 4 and 5). There was a definite pattern of wing development in time at 32°.

The same series can be demonstrated at 31°. The duration for the males at 31° and 32° was 48 hours but the rate at 31° was much slower. Consequently the mean wing attained at the end of the critical period was between the two wings shown for males at 32° of 100 and 104 hours of development. The period for the females at 31° was much shorter than that at 32° and the wings only reached a value between those of 88 and 92 hours for the males at 32° (figure 7). Changes in duration and rate with temperature changes had not affected the fundamental pattern of wing development; they simply determined the extent of the expression of that pattern in space. These changes in wing form with changes in duration or rate, together with the sequence of wing forms in time shown in figure 7, are in complete agreement with our hypotheses stated above and advanced recently (1935).

DISCUSSION

The facts established by the data presented here are: (1) beginning 1° below the critical temperature and extending through 29°, 30°, and 31° there were no significant changes in the duration of the embryonic, egg-larval or egg-larval-pupal periods; (2) their durations were practically identical for both sexes and all three temperatures; (3) the beginning of the critical period affecting wing formation at 30°, 31°, and 32° for the males and the females was the same (approximately 64 hours of total development); (4) the termination of this critical period varied with the sex and the temperature, its duration for the males being 20 hours at 30° and 48 hours at 31° and 32°, and for the females 8 hours at 30°, 20 hours at 31° and some undetermined time greater than 70 hours at 32°; (5) the rate of the developmental reactions involving the wings changed with temperature, that

is, the duration for the males at 31° and 32° was 48 hours but the maximum rate at 32° was higher than that at 31° resulting in a much larger wing at 32°. With a constant developmental period for the whole organism, and a constant point in that period for the inception of the reactions involving the development of the wings, there were changes in both the duration and the rate of these reactions with changes in temperature. Obviously we are dealing here with an example of a differential effect of temperature on various, as yet undetermined, developmental processes. (6) From the wing types produced by homozygous vestigial, homozygous dimorphos vestigial, homozygous pennant, heterozygous pennant/vestigial, and dimorphos/dimorphos pennant/vestigial through the range of 16° to 32° and from the wings produced in the temperature transfers just discussed it has become evident that (a) there is a definite pattern of wing development in time, (b) the degree of expression is dependent on the duration and rate of processes occurring in the larval period of the vestigial genotype, and (c) apparently mutations at the vestigial locus affect the duration and rate of these developmental processes. Any attempted general interpretation of these facts must fit both the data presented here and the results of STANLEY (1931, 1935).

STANLEY (1931) reported on the temperature-effective periods at 17° and 27° C. for vestigial wing and recently (1935) on those at 30° and 31° for vestigial wing and 17°, 27°, and 30° for the wild type normal allele of vestigial wing together with the heterozygotes at 17° and 27° C. With two temperatures common to his work and the data presented here certain comparisons can be made and conclusions may be drawn. Both his work (1931) and mine (1930a) show the same critical temperatures for the vestigial wings of the males and for the wings of the females. But there are significant differences for wing length at the higher temperatures resulting in somewhat different curves; differences on the whole constant and consistent in all our experiments.

His wing length for total development of males at 29° was 0.98 mm and mine 0.74 mm; at 30° we obtained 1.52 mm and 1.00 mm respectively; and at 31° we differed by 1.64 mm and 1.70 mm. The same differences were again consistently present in the maximum mean wing length for the males in his transfer experiments from 30° and 31° to a lower temperature and in the data presented here for the transferred males at those temperatures. Similar consistent differences throughout were evident for the females. My own stock was inbred and selected, STANLEY'S stock was inbred but unselected. It is obvious, I believe, that we were dealing with different sets of sex-linked and autosomal genes modifying the action of the vestigial gene. The validity of such an assumption is indicated by STANLEY'S selection and tests at the end of his experiments (1935). He

found that some of his heterozygous Wild/vestigial flies had "beaded" wings. From his original inbred and unselected vestigial S V A line he selected two new lines, S V B comparatively free of the modifying genes, and S V C practically homozygous for them. These three vestigial stocks were then tested at 29.5° and found to be significantly different in wing length. He says "Since 29.5° is very close to the 'critical temperature' for wing length, two assumptions are possible: (1) the critical temperature is shifted upward by the presence of the factors, or, (2) the factors have a direct inhibitory effect upon wing length. It is impossible, with the data available, to say which of these two assumptions is nearer the truth." In his case either may be correct. We have shown recently (HARNLY and HARNLY 1935) that the critical temperature of vestigial may be lowered as much as 5° by the presence of the sex-linked modifier dimorphos. From the consistency of the values obtained throughout his work and throughout mine and the evidence advanced for the presence of modifying genes we may assume that those points in which our results differ were due to the presence of different sets of modifying genes.

In view of those differences it is significant that we are in practical agreement on the moment in development at which temperature becomes operative. We have both found the first indications of this action in the vestigial flies (males and females) around 64–68 hours of development at 30° and 31° and I have found the same point at 32°. STANLEY reports 68 hours as the critical point at 30° for homozygous Long-winged flies. The similar time of inception of the critical period in development for the wings at these temperatures in the homozygous normal allele of vestigial and in homozygous vestigial with either of two sets of modifying factors would all indicate that its initiation was probably not due so much to the vestigial gene or its normal allele as to the reaching of some general stage or process in larval development. From CHEN's work (1929) this stage morphologically cannot be either the first appearance of the dorsal mesothoracic buds, which comes much earlier in development, nor the first appearance of the wing buds which comes much later in ontogeny. The work of ALPATOV (1929, 1930a) on the development of wild and vestigial larvae indicates that the most probable morphological point of attack is the molt between the second and third instars. Tentatively then I am assuming that the critical period in wing development at these high temperatures is initiated in time by the molt from the second to the third instar. This assumption has one major advantage over the others that might be advanced, the facility with which it may be proved or disproved. By the use of a new technique it is hoped to substantiate or invalidate this assumption in the determination of the temperature-effective period for pennant, a new regressive mutation from vestigial to a recessive wild or normal type wing.

Our close agreement on the beginning of the temperature-effective period together with both the marked differences in our end points in time at 30° and 31°, and the pronounced changes I have found in the time of termination at 30°, 31°, and 32°, indicate that the beginning and end of this period were determined by different and independent processes. Furthermore, as I have shown above, the processes ending this critical period were not associated with the effects of temperature on the general rate of development. Without any change in either the length of the egg-larval period or the time at which the temperature-effective period began at 30° and 31°, there was an increase of 140 percent in the duration of the critical period with this rise of 1° for the males of our vestigial stock. Similar results were obtained for the females. This rise of 1° in some way enabled the vestigial gene to continue its activities much longer in association with the rest of the gene complex controlling those processes leading to wing development. This interpretation agrees with the assumption that the vestigial mutation produced a gene operating within the refractory temperature limits for a much shorter interval of time and a different rate from its normal allele, consequently producing during ontogeny less of the precursor of the ultimately formed wing bud. The differences in the termination points and the duration of the egg-larval periods as related to the duration of the temperature-effective periods determined at 30° and 31° by STANLEY and myself were probably due to the different sets of modifiers present in our vestigial stocks. That even a single modifier may have a profound effect on the action of the vestigial gene was shown by the fact that the mutant sex-linked modifier dimorphos enabled the vestigial gene to produce wild-type wings at these high temperatures (HARNLY and HARNLY 1935). We may conclude that the initiation of the temperature-effective period at these high temperatures was dependent on the attainment of some developmental stage (probably the molt from the second to the third instar) and that its termination was independently determined by the response of the wing gene complex to specific temperatures, the consequent durations in my stock being disproportional to the duration of the larval periods in which they occurred. The rates of the processes concerned with wing formation were likewise dependent on or related to the temperature experienced during the critical period of development. Any attempt to explain further the action of the vestigial gene and its relation to temperature would be hazardous at this time. The responses of its allele pennant and the heterozygote pennant/vestigial in total development through the temperature range of 16° to 32° preclude any explanation in terms of the data available at present. These experiments with pennant and pennant/vestigial have been completed and will be reported shortly.

GOLDSCHMIDT in the course of his extensive studies on *Lymantria dispar* presented in 1920 a general theory of the action of the genes in development, a theory which he has evolved more completely since then (GOLDSCHMIDT 1934, complete bibliography). This theory is based on his work on intersexuality and pigmentation patterns in the larvae and assumes that the genes affect the rates and durations of processes in ontogeny. The data presented here on vestigial are in agreement with his hypothesis.

SUMMARY

1. The temperature-effective periods for the length and area of the vestigial wings of an inbred selected stock of *D. melanogaster* are reported for 30°, 31°, and 32°.

2. The critical period began at approximately 64 hours for both sexes at these temperatures. It had a duration of 20 hours at 30° and 48 hours at 31° and 32° in the males and 8, 20, and some interval more than 70 hours for the females. Rises of 1° at these temperatures did not affect the inception of the period but markedly affected its termination and the rate; that is, in the males at 31° and 32° with no change in duration there was a marked increase in the rate.

3. The differences between the sexes in duration and rate with increases of 1° afford a formal explanation of the sexual dimorphism of the wings.

4. A complete reversal of the relative sizes of the wings of the males and the females was found at 33° for all developmental points examined.

5. There was never any close approach to the wild type wing at these high temperatures. The growth pattern of wing form in time is shown and related to the alleles at the vestigial locus and the theory of wing pattern and mutation stated in an earlier paper.

6. The duration of the embryonic, egg-larval, and egg-larval-pupal periods were determined at 25°, 29°, 30°, 31°, and 32°. Pronounced differences in the length of these periods were found between 25° and 29°. No significant changes occurred in their duration at 30° and 31°. The effects of temperature above 29° on the duration of the major periods of development offer no explanation of the critical temperatures, the sexual dimorphism, or the increase in wing size. The results must be due to a differential effect of temperature on various processes during ontogeny.

7. The inception of the critical period in development is interpreted as occuring at the molt from the second to the third instar at these high temperatures; its termination is apparently independently determined by the gene complex (including sex) and the temperature experienced, the consequent durations being disproportional to the durations of the larval periods. The rate is likewise dependent on the gene complex and the temperature.

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THE INHERITANCE OF SUSCEPTIBILITY TO YELLOW FEVER ENCEPHALITIS IN MICE

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INTRODUCTION

THE importance of host resistance as a factor in determining the incidence of infectious disease has been emphasized in recent studies. The methods developed in the study of genetics have provided a practical means of investigating the problems of host resistance and from the accumulated data there can be no doubt of the existence of heritable factors which affect the response of the individual to infection. A bibliography of the more important contributions in this field is given in a review of the subject by HILL (1934). The experiments to be reported here give evidence of the presence of such factors influencing the incidence of a virus disease, yellow fever.

The susceptibility of mice to the virus of yellow fever was demonstrated by THEILER (1930). SAWYER and LLOYD (1931) tested the suitability of mice as test animals in the study of yellow fever immunity and found that different strains of mice varied in susceptibility to this virus, ranging from 100 percent to 50 percent mortality with standard dosage of virus. This information was not only of practical value in the work on yellow fever immunity, but also indicated the operation of genetic factors in yellow fever resistance. On this basis the following investigation was undertaken.

METHOD OF TESTING

Since yellow fever infection occurs in mice only as an encephalitis, test inoculations were made by intracerebral inoculation while the mice were under ether anaesthesia. Susceptible mice when so inoculated with a virus "fixed for mice" show a typical paralysis which usually occurs on the fourth or fifth day after inoculation and terminates in death within one day from onset. Mice showing no paralysis in ten days are classed as survivors and are discarded.

The virus selected had been "fixed" by passing in mice from brain-to-brain for over 100 passages and had reached its maximum virulence for this animal. To obtain approximately equal dosages, a virus was used which had been preserved by drying while in the frozen state, in which form SAWYER, LLOYD, and KITCHEN (1929) have shown its virulence to be retained over a period of years. The dried virus was diluted with a

sufficient quantity of suitable diluent to yield a final concentration in which approximately 30 minimum lethal doses were contained in 0.03 cc., which amount is inoculated intracerebrally into the mice to be tested. However, for certain technical reasons concerned with the drying of virus, tubes opened at different times vary in virus content, and since the keeping quality of the virus is such that titrations cannot be completed before the virus activity is lost, the doses are not uniform. To overcome this difficulty the contents of several tubes are pooled for each test and to demonstrate viability of the virus a number of unselected Swiss mice are inoculated with each virus suspension used.

MATERIAL

The "Swiss" strain was chosen as a source of susceptible animals. The foundation stock consisted of 2 males and 7 females obtained in 1926 from Dr. A. de Coulon in Lausanne, Switzerland. The individuals used in these experiments were taken from lines, some of which were inbred brother by sister and others partly pen inbred. SAWYER and LLOYD (1931) had previously tested mice from a branch of this stock, observing 100 percent mortality in each of two lots consisting of 30 and 23 mice respectively.

TABLE 1

Mortality percentages of mice from the Swiss and Det strains. Preliminary tests

TESTED BY	GROUP	NUMBER TESTED	NUMBER DEAD	PERCENT DEAD	PERCENT DIFFERENCE	χ^2	P
Sawyer and Lloyd	Swiss (test 4)	30	30	100.0			
	Swiss (test 5)	23	23	100.0	18.5	10.83	<.01
	Det (test 3)	54	44	81.5			
Lynch and Hughes	Det	88	58	65.9	15.6	4.01	.05
	Swiss	138	134	97.1	31.2	40.91	<.01

The strain selected as a source of resistant stock had also been tested by SAWYER and LLOYD. Although in their tests the lowest mortality had been given by the "Rockefeller Institute Stock," certain practical considerations led to the choice of stock referred to by SAWYER and LLOYD as "Strain D" and designated by us as "Det." Five pairs were purchased and they and their descendants were inbred, usually brother by sister, but occasionally parent by offspring. Mice from the second to the sixth generations were used in these tests.

In the hands of SAWYER and LLOYD, the Det mice had a mortality of 81.5 percent (table 1), about 18.5 percent lower than the Swiss strain; however, the comparison may not be accurate since the two groups were

inoculated with different virus preparations. A preliminary test of the Det mice in our colony resulted in a distinctly lower figure: of 88 mice inoculated, 65.9 percent succumbed. Among 138 Swiss mice tested with the identical virus preparation, there was 97.1 percent mortality. The difference is 31.2 percent and the χ^2 test for the significance of the difference gives P as $<.01$. This result confirmed our choice of material since the divergence of the strains is sufficiently great to warrant a cross between them. Therefore matings were made between representatives of the Det and Swiss strains and the hybrids thus produced were crossed back to the parental strains, sometimes to the original parents. The resulting population was divided into two lots and was inoculated with virus. Variations in the rate of reproduction together with exigencies of time and laboratory space prevented a regular scheme of distribution into two groups so parents and their offspring were not always inoculated together. During the course of the experiment some animals died of intercurrent disease before their reaction to yellow fever virus could be determined. Not all individuals of the P_1 and F_1 generations contributed offspring to the experiment.

RESULTS

The first test included samples from all the generations under observation (table 2). The original strains were represented by 39 Swiss and 18 Det mice. Upon inoculation all the Swiss mice succumbed, while the

TABLE 2

Test I. Mortality percentages of representatives of the parental strains, F_1 and backcross progeny

GROUP	NUMBER TESTED	NUMBER DEAD	PERCENT DEAD	PERCENT DIFFERENCE	χ^2	P
Swiss	39	39	100.0	22.2	9.32	$<.01$
Det	18	14	77.8			
F_1 (Swiss×Det)	37	31	83.8			
Backcross (F_1×Swiss)	146	137	93.8	23.1	24.03	$<.01$
Backcross (F_1×Det)	99	70	70.7			

mortality rate of the Det mice was 77.8 percent. The difference between the strains, when measured by the χ^2 test is significant, $\chi^2 = 9.32$ and P $= <.01$. Thirty-seven individuals were available for examination in the F_1 generation obtained from the cross Swiss by Det. Of these 31, or 83.8 percent died—a mortality not much higher than that of the Det and significantly different from that of the Swiss strain ($\chi^2 = 6.86$; $P = <.01$). In the backcross generation somewhat larger numbers are dealt with, the two classes comprising 146 and 99 animals respectively. The descendants produced by crossing the hybrid to the more susceptible strain showed a heightened mortality (93.8 percent) while the mice resulting from the

backcross to the less susceptible stock showed a decreased mortality (70.7 percent). This difference is greater than would be expected on a basis of random sampling of the same population, having a χ^2 value of 24.08 and $P = <.01$.

The second test also included representatives of the five classes (table 3). Only 8 Swiss mice were available but again the mortality was 100 percent, while 61 mice from the Det strain showed only 50.8 percent mortality—the lowest rate yet observed. The F_1 had a mortality of 65.4 percent. Compared with the Swiss strain the difference in mortality is probably significant ($\chi^2 = 3.76$; $P = .05$). The backcross progeny produced by mating the F_1 hybrids to the more susceptible strain showed a mortality of 70.2 percent while in the backcross to the less susceptible strain the mortality was reduced to 50.0 percent. The difference between them is 20.2 percent, about the same as in the first experiment, and is also mathematically significant ($\chi^2 = 8.32$ and $P = <.01$).

TABLE 3

Test II. Mortality percentages of representatives of the parental strains, F_1 and backcross progeny

GROUP	NUMBER TESTED	NUMBER DEAD	PERCENT DEAD	PERCENT DIFFERENCE	χ^2	P
Swiss	8	8	100.0	49.2	6.96	<.01
Det	61	31	50.8			
F_1 (Swiss×Det)	26	17	65.4			
Backcross (F_1×Swiss)	114	80	70.2	20.2	8.32	<.01
Backcross (F_1×Det)	84	42	50.0			

In comparing the two tests certain similarities are apparent. In both cases there is a significant difference between the susceptible and partially resistant stocks, the hybrids are more like the resistant strain and the backcross groups have a heightened or lowered mortality depending on the degree of susceptibility of the parent stock to which the backcross was made. On the other hand, the dissimilarities are so marked that it becomes doubtful whether the figures of the two tests should be combined. Each class in the second test has a lower mortality than its corresponding set in the first test, with the exception of the Swiss in which only 8 individuals were used in the second test. The samples of the parental Det strain gave significantly different mortalities in the two tests and the backcross progeny from the susceptible strain showed no greater mortality in the second test than did the backcross progeny from the resistant strain in the first test. Since in all probability the quantity of virus in the inoculum varied between tests, the lower mortality rate in the second test may reasonably be referred to this difference in virus content. However, a further genetic analysis of the data is possible. Some of the hybrid parents were back-

crossed in the two directions. We find that, in the first experiment 4 F_1 parents, and in the second test 6 F_1 parents were mated with individuals from each stock. Their offspring are listed in tables 4 and 5. These tables

TABLE 4

Test I. Mortality rates of the progeny of the same F_1 individual backcrossed to both parental strains

IDENTIFICATION NUMBER OF F_1 PARENT	BACKCROSS PROGENY				SIGNIFICANCE OF DIFFERENCE
	FROM F_1×DET		FROM F_1×SWISS		
	NUMBER DEAD	NUMBER ALIVE	NUMBER DEAD	NUMBER ALIVE	
♂ 69.0	8	6	14	0	
♂ 70.0	5	2	22	1	
♀ 54.1	5	1	6	0	
♀ 54.3	4	0	5	0	
Total	22	9	47	1	$\chi^2=12.37$ P= <.01
Percent dead	71.0		97.9		

show in detail the degree of regularity with which these individuals produced offspring with unlike mortality ratios among siblings resulting from contrasted matings. The totals again indicate hereditary differences.

TABLE 5

Test II. Mortality rates of the progeny of the same F_1 individual backcrossed to both parental strains

IDENTIFICATION NUMBER OF F_1 PARENT	BACKCROSS PROGENY				SIGNIFICANCE OF DIFFERENCE
	FROM F_1×DET		FROM F_1×SWISS		
	NUMBER DEAD	NUMBER ALIVE	NUMBER DEAD	NUMBER ALIVE	
♀ 68.20	0	5	10	0	
♀ 55.40	1	2	3	0	
♀ 55.25	1	4	7	5	
♀ 61.46	0	11	10	2	
♂100.00	6	12	9	8	
♂101.00	1	0	6	3	
Total	9	34	45	18	$\chi^2=26.08$ P= <.01
Percent dead	20.9		71.4		

A more exact comparison may be made between the offspring of the various types of mating when the susceptibility of both parents is known. The animals for which this information is available are grouped in table 6. In the first test all of the immediate progeny in any generation resulting

from crosses between two susceptible parents may be compared with the progeny resulting from matings in which only one parent was susceptible. There were only 9 mice available in the second class but their mortality was 22.2 percent as contrasted with a mortality of 91.4 percent among those with two susceptible parents. In the second test the figures for the corresponding classes are 27.6 percent and 69.6 percent, again a significant difference. In addition there was a third class comprised of 15 individuals with two resistant parents. In this group there was but one death, a mortality rate of 6.7 percent; however, this figure is not significantly lower than that given by the offspring from unlike parents.

TABLE 6

Mortality percentages among mice classified as to whether both parents were susceptible, only one susceptible, or both resistant

TEST	PARENTAGE	NUMBER DEAD	NUMBER ALIVE	PERCENT DEAD	PERCENT DIFFERENCE	χ^2	P
I	S×S	74	7	91.4	69.1	29.47	<.01
	S×R	2	7	22.2			
II	S×S	16	7	69.6	42.0	9.10	<.01
	S×R	8	21	27.6			
	R×R	1	14	6.7	20.9	2.66	.10

S = Susceptible.
R = Resistant

By this classification also the values obtained in the two tests are not similar. In the first test the mortality in the population derived from susceptible parents was 91.4 percent while in the second test it was only 69.6 percent. Since it has not been shown that the parental matings were between homozygotes, the genetic expectation for these values cannot be computed. However, as a whole the data seem sufficient to justify the conclusion that the type of parentage and the susceptibility of the offspring are correlated.

On the assumption that the two tests may properly be treated as self-contained units, an inquiry into the homogeneity of the groups of which they were composed was undertaken. The Lexian ratio was used to test the dispersion of the probabilities of death among the litters in each group, although the numbers dealt with in some cases were small. In the preliminary study of the Swiss, the sibling relationships were known for only 96 mice but this number which contained the only four resistant animals of the group proved homogeneous (table 7). While the Det stock appeared uniform in the first test, all of the mice used as progenitors were not tested and the larger population used in the second test was not homo-

geneous. The F_1 hybrid generation gave different results in the two tests. The backcross progenies lacked uniformity but homogeneity was shown in the groups classified on a basis of parentage.

TABLE 7

Tests for the type of dispersion of the probabilities of death within various groups

TEST	GROUP	NUMBER TESTED	NUMBER OF FAMILIES	L	χ^2	P
Preliminary	Det	88	27	1.22	40.27	.04
	Swiss	96	35	1.10	42.59	.18
I	Swiss	39	—	—	—	—
	Det	18	6	1.11	7.39	.19
	F_1 (Swiss×Det)	37	7	1.41	13.85	.04
	Backcross (F_1×Swiss)	146	20	1.71	59.06	<.01
	Backcross (F_1×Det)	99	20	1.52	45.94	<.01
II	Swiss	8	—	—	—	—
	Det	61	25	1.35	45.60	<.01
	F_1 (Swiss×Det)	26	7	1.12	8.73	.19
	Backcross (F_1×Swiss)	114	22	1.35	40.27	.01
	Backcross (F_1×Det)	84	22	1.63	58.53	<.01
I	S×S	81	13	0.77	7.66	.81
	S×R	9	2	0.40	0.32	.59
II	S×S	23	5	1.16	6.84	.15
	S×R	29	8	1.01	8.14	.32
	R×R	15	3	1.20	4.29	.12

In table 8 the various groups are arranged according to sex. There is no indication that sex is a differentiating factor. The apparently greater susceptibility of females shown in the last backcross group where a test

TABLE 8

Comparison of mortality of the sexes within various groups

TEST	GROUP	SEX	NUMBER DEAD	NUMBER ALIVE
I	F_1 (Swiss×Det)	♂♂	15	4
		♀♀	17	1
	Backcross (F_1×Swiss)	♂♂	55	3
		♀♀	82	6
	Backcross (F_1×Det)	♂♂	30	16
		♀♀	40	13
II	F_1 (Swiss×Det)	♂♂	10	6
		♀♀	7	3
	Backcross (F_1×Swiss)	♂♂	44	19
		♀♀	36	15
	Backcross (F_1×Det)	♂♂	12	25
		♀♀	30	17

for independence gives χ^2 as 8.16 and P as <.01, is probably due to the fact that in several litters where resistance was high there were no sisters brought to test.

DISCUSSION

In attempting to demonstrate genetic factors, it becomes necessary to control as far as possible any environmental conditions which may affect the results of the tests. The immediate surroundings in the laboratory were kept as constant as was possible, but many variables exist which are beyond control. In this experiment the occurrence of acquired immunity may safely be ruled out, since the mice were sent to a laboratory remote from the breeding room for tests. No survivors were returned. There can be no question as to the freedom of the tested animals from previous contact with yellow fever virus.

No selection of strains was undertaken during this experiment. Rather, the investigation was planned to take advantage of strains from diverse sources in which some differentiation already had occurred. Although lack of known material for the various crosses precludes a final analysis, the existence of segregating factors for susceptibility and resistance is evident. This is clearly shown by the differential ratios given first by backcrosses and second by progeny from different sorts of matings. The latter criterion for inheritance is not of universal application. When in a genetically pure strain, the character in question, because of somatic fluctuation, does not manifest itself with absolute regularity, progeny from a mixed parentage do not differ significantly from offspring descended from like parents. If progeny groups are dissimilar, whether the parents do or do not present phenotypic differences, they must be different genetically. Consequently progeny are the most reliable basis for determining the constitution of parents. When differing groups of progeny are descended from unlike types of matings, as in the present case the genetic explanation is self-evident.

As to the mode of inheritance, since matings between like parents, either both susceptible or both non-susceptible, produced two types among their offspring either fluctuating variations or the joint occurrence of a number of genes are indicated. Neither of these alternatives can be ruled out by our data.

SUMMARY

1. Mice from two sources gave different mortality rates when inoculated with the virus of yellow fever. This difference was maintained in three separate tests.

2. When the strains were crossed, the hybrids showed a mortality less than that of the susceptible strain. In one test the difference was clearly significant; in another it was probably significant. By crossing the hybrid back to the susceptible strain the mortality rate was increased; by crossing back to a more resistant strain the rate was lowered. This relationship was demonstrated in two tests.

3. When the mice were classified according to parentage, offspring from two susceptible parents were more susceptible than were offspring with one or two resistant parents.

4. Susceptibility did not appear to be modified by sex.

5. It is concluded that hereditary factors for resistance to yellow fever encephalitis are present in mice.

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STUDIES ON HYBRID STERILITY. II. LOCALIZATION OF STERILITY FACTORS IN DROSOPHILA PSEUDOOBSCURA HYBRIDS

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THE PROBLEM

THE causation of hybrid sterility has long been one of the unsolved problems of biology. To date, probably the greatest advance in this field has been made by FEDERLEY who discovered a failure of the meiotic chromosome pairing in the sterile hybrids between moths of the genus Pygaera. This finding has since been amply corroborated by other investigators in sterile hybrids both in animals and in plants. Naturally enough it became tempting to suppose that the failure of the meiotic pairing is the cause of hybrid sterility. The restitution of the normal meiotic pairing as well as of fertility following the reduplication of the chromosome complement in allotetraploid hybrids seems to be further evidence in favor of this view. And yet, this view proves to be inadequate as a general explanation of hybrid sterility. Two difficulties deserve particular attention. First, some hybrids are sterile despite the fact that the meiotic pairing in their gametogenesis is apparently normal (MICHAELIS 1933 in Epilobium, DOBZHANSKY 1934 in some crosses in *Drosophila pseudoobscura*); while in other sterile hybrids the gametogenesis does not reach the meiotic stages (KERKIS 1933, in *Drosophila melanogaster* × *D. simulans*). Second, the failure of the meiotic pairing in sterile hybrids is usually attributed to an "incompatibility" of the chromosomes of species or races entering the cross. This, clearly, is a restatement of facts and not a causal explanation. It remains possible that suppression of meiotic pairing may be caused by different mechanisms in different cases, and that sometimes there is no cause and effect relation between the failure of pairing and the sterility.

In my previous publications (DOBZHANSKY 1933, 1934) a hypothesis was suggested according to which there exist at least two different types of hybrid sterility—the chromosomal type and the genic type. The chromosomal type is caused by differences in the gross structure (gene alignment) of the chromosomes of the parental forms, preventing, through competition in pairing, the normal conjugation of the chromosomes at meiosis, and causing their irregular disjunction. The genic type of sterility is due to interactions between complementary genetic factors contributed by both parents. If the genetic constitution of one of the parental forms is *SStt*, and of the other *ssTT*, the hybrid is *SsTt*. The assumption is made that the presence of the factor (or the group of factors) *S* alone, or of the

factor *T* alone, permits unlimited fertility, but that the factors *S* and *T* interact in such a manner as to make sterile an organism carrying them simultaneously.

Although some indirect evidence for the existence of these two sterility producing mechanisms is available (cf. DOBZHANSKY 1934), the above hypothesis cannot yet be considered proved. To date, in no case has a sufficient amount of gross structural differences between chromosomes of related species been demonstrated, nor have the theoretically postulated sterility genes been isolated and localized. The experiments to be reported in the present article are aimed at securing some information on this subject. The sterility of the male hybrids between race A and race B of *Drosophila pseudoobscura* is apparently of the genic type, (DOBZHANSKY and BOCHE 1933, DOBZHANSKY 1933, 1934). The results of LANCEFIELD (1929) and of KOLLER (1932) suggest that some of the differences producing sterility between these races are located in the X-chromosome, while autosomes seem also to be involved (DOBZHANSKY 1933a). Further attempts to localize the sterility genes in the chromosomes of *Drosophila pseudoobscura* are described below.

The writer wishes to acknowledge his obligations to Professors C. V. BEERS (Los Angeles), F. A. E. CREW and P. C. KOLLER (Edinburgh) for permission to use some of the mutants discovered by them in *D. pseudoobscura* the descriptions of which are not yet published. Thanks are due also to Doctor A. H. STURTEVANT for many valuable suggestions and criticisms.

METHOD

Factors whose interaction is responsible for the sterility of a hybrid are a part of the germ plasm, and hence must have a physical carrier in the gametes. The task is to find out in which of the constituents of the gametes these factors are localized. Races A and B of *D. pseudoobscura* produce in F_1 completely sterile male hybrids, but fertile females. The latter may be back crossed to males of either parental race. In the offspring of the backcrosses various combinations of the chromosomes and of the cytoplasms of the ancestral forms should occur in separate individuals. Some of the individuals may be expected to be sterile, and others fertile. Provided the chromosomes are marked by appropriate mutant genes, the genetic structure of a given individual may be recognized by its phenotype. Hence, it will be possible to determine which combinations of the ancestral elements are necessary to induce sterility, and which permit the individual to be fertile.

In practice, two experimental procedures are possible. First, by repeatedly backcrossing hybrid females to males of the same race, one may secure individuals carrying one of the elements (e. g., a chromosome or a

part thereof, or the cytoplasm) of one race, and all other elements from the other race. Second, among the male offspring of the first backcross generation individuals should be found representing many of the possible combinations of the above elements. Both experimental procedures should, probably, be made use of before a final solution of the problem here under consideration may be consummated. The data reported in the present article are based on the results of the application of the second procedure. The conclusions arrived at are, however, in accord with those suggested by the outcome of the few experiments of Doctor A. H. STURTEVANT and the writer involving the application of the first procedure.

Males appearing in the progeny of the backcrosses have testes ranging in size from normal (i.e., that found in males of either parental race) to very small. Males having small testes are invariably sterile, those with testes of normal or nearly normal size are mostly fertile. It has been shown cytologically (DOBZHANSKY 1933, 1934) that the disturbances of the spermatogenesis leading to sterility are greatest in the very small testes, and least in large ones. Hence, size of the testis is a fair measure of the degree of departure from the normal structure and functioning of the testis. This fact is very important for our purposes, since it permits a sort of quantitative expression of the results of investigation of the hybrid males. The smaller the testes in males of a given genetic constitution, the more (or the stronger) sterility factors they carry.

TECHNIQUE

Testis size in *D. pseudoobscura*, particularly in the hybrid males, is exceedingly sensitive to environmental factors. Although our conclusions are to be based on a comparison of testis size in different classes of males developed in the same culture bottles, and hence under identical environment, care was taken to insure homogeneous culture conditions in all experiments. Three to four F_1 hybrid females were placed in the same bottle with five to six males. Parents were kept in vials with food for four days, transferred (without etherization) to standard culture bottles, placed in incubator at 24.5° C., and allowed to oviposit for about four days, transferred to fresh culture bottles, and again left there for a similar length of time.

When the progeny of the backcross started to emerge, flies were classified according to the marking genes they carried, and males were dissected in physiological salt solution. Testes were isolated, and their length measured with the aid of an eyepiece micrometer (1 unit = 17.4μ). The technique of the measurements has been described by DOBZHANSKY and BOCHE (1933). The testis of *D. pseudoobscura* is ellipsoidal in shape; its greater diameter shows a correlation with its shorter diameter, so that either of

these measurements gives a fair idea of the size of the whole organ. However, in the adult males the shape of the testis changes somewhat with age, becoming relatively longer and more slender. To avoid this source of error, cultures were examined every second day, and males that had hatched in the meanwhile were dissected and measured immediately. Only males that emerged during the first 6 to 8 days of hatching in a given culture bottle were used.

The statistical data obtained are far too voluminous to be published in detail under the present conditions; they are, of course, preserved, and open to all interested. The statistical constants were calculated only for classes in which ten or more individuals were measured.

PRELIMINARY EXPERIMENTS

Theoretically, sterility factors might be located in any of the constituents of the gametes. A few simple experiments of exploratory nature were undertaken to narrow the range of possibilities by excluding some of them as inadequate to explain the situation.

If F_1 hybrid females from the cross A♀ × B♂ are used for making backcrosses, the resulting progeny has race A cytoplasm. By crossing such females to race B males, some of the males appearing in the next generation should have all race B chromosomes in race A cytoplasm. If the sterility depends upon an interaction of the chromosomes of one race with the cytoplasm of the other, such males should be sterile. Actually in a number of tests they were found to be fertile. Similarly, males having race A chromosomes in race B cytoplasm proved fertile. This shows that at least the most important sterility factors are not located in the cytoplasm.

To exclude the influence of the Y chromosomes is more difficult. The males devoid of the Y chromosome (XO males) in pure races are sterile, but the structure of their testes shows no similarity whatever with that of the sterile hybrid males (DOBZANSKY 1933). This is in agreement with the results of SHEN (1932) who found that the sterility of XO males in *D. melanogaster* is caused by disturbances in the vesiculae seminales rather than in the testes. Some hybrid males in the backcrosses of hybrid females to race A males are likewise XO. These are, of course, always sterile, but the size, as well as the internal structure of their testes, is variable, just as in their XY sibs. This variability can be due only to the chromosomes other than the Y carried by a given male (DOBZHANSKY 1933). This makes the assumption of factors responsible for hybrid sterility in the Y chromosome unnecessary.

LANCEFIELD (1929) and KOLLER (1932) found that males carrying an X chromosome of one race and a majority of the autosomes of the other race have small testes and are sterile. Furthermore, by studying crossovers

in the X chromosome they found that both ends (or, rather, both limbs) of the X chromosome are concerned with the sterility, while the middle part is apparently not involved. Thus the location of some of the sterility factors was established.

STURTEVANT and the writer attempted to "transfer" mutant genes known in one race into the other race. For this purpose females carrying a given gene, for instance of race A, are repeatedly backcrossed to males of race B, until individuals are obtained possessing all chromosomes of race B except for a more or less short section containing the genes in question. These experiments showed that some of the mutant genes can be thus transferred from race to race, and males carrying these "foreign" genes become fertile after from one to several generations of backcrosses. To this class belong the genes Pointed and short (in the X chromosome of race A), Smoky (second chromosome of race B), Curly (fourth chromosome of B). It follows that sections of chromosomes including these genes contain either no factors concerned with sterility, or else these factors are not by themselves sufficient to produce sterility (although they may, perhaps, do so in combinations with other genes). On the other hand, the attempts to transfer the genes Bare (A) and cinnabar (B) (second chromosome) from one race to another have so far given negative results despite the numerous backcrosses. Similarly, Pointed proved to be closely linked with a sterility-producing section of the X chromosome, but it can be separated from this section by crossing over. Thus, sterility-producing genes were found in both the X chromosome and in an autosome (the second chromosome). The data presented below corroborate this conclusion.

Backcross to race A

Race A females carrying the sex-linked recessives beaded (*bd*), yellow (*y*), and short (*s*), the second chromosome dominant Bare (*Ba*), and the third chromosome recessive purple (*pr*) were crossed to race B males carrying the third chromosome recessive orange (*or*) and the fourth chromosome dominant Curly (*Cy*). F_1 hybrid females heterozygous for these genes were selected and backcrossed to race A males homozygous for *or* and *pr*. The males coming from this backcross were classified for all of the above genes.

According to the setting of the experiment, every one of the chromosomes of the F_1 females (with the exception of the small fifth chromosome in which no genes are available) is marked by one or more genes, which should make the different classes of the backcross males distinguishable from each other phenotypically. Unfortunately, the control of all the chromosomes which is thus attained is far from complete, due to crossing over which takes place in the hybrid females. The X chromosome is

marked by three genes, the third by two, and the second and fourth by only one each. The scarcity of marking genes is mitigated by the fact that the X, second, and third chromosomes of race A differ from the corresponding B chromosomes by inverted sections (TAN 1935 a, b) which suppress a part of the normal crossing over. Nevertheless, some crossovers undoubtedly escape detection.

TABLE 1

Length of the testis (in μ) in the offspring of the cross: (bd y s Ba pr Race A ♀ × or Cy Race B ♂) F₁ ♀ × or pr Race A ♂.

PHENOTYPE	M±m	n	PHENOTYPE	M±m	n
1 *bd y s Ba pr*	668.2	9	33 *bd y Ba pr*	348.0	1
2 *bd y s Ba pr Cy*	563.8	8	34 *bd y Ba py Cy*	342.8	3
3 *bd y s Ba or*	585.9±14.9	23	35 *bd y Ba or*	478.5	6
4 *bd y s pr*	632.8±11.0	30	36 *bd y pr*	504.6	4
5 *bd y s Ba or Cy*	509.6±17.6	19	37 *bd y Ba or Cy*	407.2	9
6 *bd y s pr Cy*	602.7± 3.4	23	38 *bd y pr Cy*	435.0	3
7 *bd y s or*	551.2± 6.1	119	39 *bd y or*	462.8	8
8 *bd y s or Cy*	526.5± 8.9	66	40 *bd y or Cy*	418.1±24.4	17
9 *or Cy*	123.9± 2.8	336	41 *s or Cy*	290.2±18.1	22
10 *or*	113.1± 3.2	353	42 *s or*	310.9±18.6	27
11 *pr Cy*	94.0± 3.6	152	43 *s pr Cy*	231.4	4
12 *Ba or Cy*	66.6± 4.7	69	44 *s Ba or Cy*	191.4	1
13 *pr*	68.4± 3.7	159	45 *s pr*	187.9	5
14 *Ba or*	60.2± 4.6	71	46 *s Ba or*	34.8	1
15 *Ba pr Cy*	51.7± 5.9	38	47 *s Ba pr Cy*		
16 *Ba pr*	23.8± 7.3	23	48 *s Ba pr*		
17 *bd y s Ba*	640.3	5	49 *bd y Ba*	461.1	2
18 *bd y s Ba or pr*	664.7	4	50 *bd y Ba or pr*		
19 *bd y s Ba Cy*	553.3	5	51 *bd y Ba Cy*		
20 *bd y s Ba or pr Cy*			52 *bd y Ba or pr Cy*		
21 *bd y s*	597.2±10.4	22	53 *bd y*	574.2	1
22 *bd y s or pr*	617.7±18.4	10	54 *bd y or pr*	614.2	3
23 *bd y s Cy*	559.8±15.6	24	55 *bd y Cy*	461.1	2
24 *bd y s or pr Cy*	494.2	6	56 *bd y or pr Cy*	626.4	1
25 *Cy*	122.8± 4.8	82	57 *s Cy*	274.9	4
26 *or pr Cy*	94.1±10.3	35	58 *s or pr Cy*	487.2	1
27 wild-type	110.7± 5.0	86	59 *s*	261.0	6
28 *or pr*	69.8 ± 6.7	33	60 *s or pr*	104.4	1
29 *Ba Cy*	43.5± 7.3	19	61 *s Ba Cy*		
30 *Ba or pr Cy*	34.8	5	62 *s Ba or pr Cy*		
31 *Ba*	17.1± 4.6	21	63 *s Ba*	87.0	2
32 *Ba or pr*	47.0	3	64 *bd or*	574.2	1

In spite of the above difficulty, it is believed that the control of the chromosomes, incomplete as it is, is on the whole adequate for our purpose, namely testing for the presence or absence of the sterility genes in each of the chromosomes covered by the investigation. The basis of our judgement is the mean testis size in the males of a given class showing a given combination of the marking genes. Although some of the chromosomes are

marked by a single gene only, all the males carrying this gene will carry at least a part of the chromosome of the race indicated by this gene, and the majority will carry probably the entire chromosome. In any event,

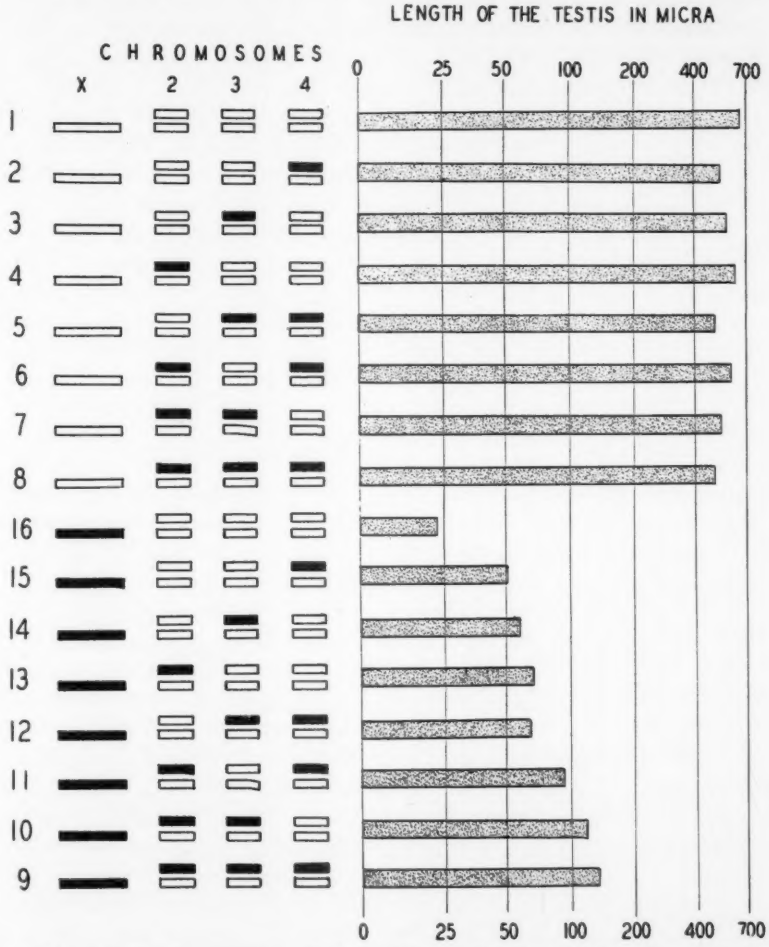

FIGURE 1. The chromosome constitution and the length of the testis in males appearing in the backcross of the F_1 hybrid females to race A males. Race A chromosomes—white; race B chromosomes—black. Only the non-crossover classes are represented.

males of every class have a greater chance to have the chromosomal constitution indicated by the marking genes they carry than any other constitution. In other words, since the conclusions are to be based on averages

and not on individual variants, the disturbing influence of the undetected crossovers will manifest itself in a great variability and not in a distortion of the relationships of different classes. These remarks apply to all the experiments described in this article.

Every individual resulting from a backcross to race A male necessarily has one whole complement of autosomes from race A. All males have also a Y chromosome from race A. However, some of them have a race A X chromosome, and others a race B X chromosome. They inherit from their mothers one or more autosomes of race A, or of race B. Not counting crossovers, and disregarding the fifth chromosome, the backcross males should fall into sixteen classes (classes 1–16 in table 1, figure 1).

Males receiving race A X chromosomes (classes 1–8, 17–24) have distinctly larger testes than those receiving race B X's (classes 9–16, 25–32). It follows that the X chromosome carries genes concerned with the sterility. Next, one may notice that different classes of males carrying the same X chromosome vary greatly as to testis size. Classes possessing the B race X chromosome may be taken up first. Among them the largest testes are observed in individuals having one full set of race B autosomes (class 9, also 25 and 26). Making such males homozygous for the race A fourth chromosome decreases testis size (compare classes 9 and 10, 25 and 27, 26 and 28). The differences are not in all cases statistically significant, but they are always in the same direction. Homozygosis for race A third chromosome also decreases testis size, the effect of the third chromosome being stronger than that of the fourth (compare classes 9 and 11). The race A second chromosome produces a still stronger effect in the same direction (compare 9 and 12, 25 and 29, 26 and 30). The third and the fourth chromosomes of race A being homozygous simultaneously produce as much effect as the second alone (12 and 13). Homozygosis for second and fourth, and second and third depresses testis size still further (14 and 15). Finally, males possessing only a race A autosomes have the smallest testes (class 16). The conclusion is warranted that in males carrying race B X chromosome the testes are larger the more B autosomes they carry (although, as stated above, all these males carry one full set of A autosomes).

The mean values for testis size in males having race A X chromosome are based on a smaller number of flies than those discussed above. Nevertheless, it is quite clear that in these males the relations are reversed, namely the testes are the larger the more race A autosomes they carry. The largest testes are observed in males carrying all or most of race A autosomes (classes 1, 17, 18), and the smallest in males having a full set of B autosomes (8, 23, 24). Hence, a general rule may be formulated thus: in the backcross males the testes are larger the more the autosomes agree

in their racial origin with the X chromosome, and vice versa. The second, third, and fourth chromosomes are all concerned, but to a different extent: the second chromosome exerts the strongest effect, the third is next, and the fourth last.

Since two of the chromosomes in this experiment contain more than one marking gene each, some crossovers are detected. This makes it possible to determine (a) whether these chromosomes contain more than one locus concerned with testis size, and (b) if this be the case, which part of a given chromosome exerts a stronger effect.

Three genes, *bd*, *y* and *s*, are present in the X chromosome. Crossing over between *bd* and *y* has been observed in our hybrids only once (class 64), although about 30 percent of crossing over takes place between these genes in pure race A (this is due to the inversions in the left limb of the X suppressing crossing over, TAN 1935). On the other hand, crossing over between *y* and *s* is fairly frequent (though less frequent than in pure race A, where these loci are practically independent). Classes carrying the whole X chromosome of race A (*bd y s*, classes 1–8, 17–24) may be compared with those having the right limb, or a part thereof, of race B (*bd y*, classes 33–40, 49–56). If this comparison is made so that classes differing only in the substitution of the right limb of the X are considered (classes 1 and 33, 2 and 34, 17 and 49, 24 and 55, etc.), the conclusion is that such a substitution decreases testis size. This is observed in twelve out of thirteen such comparisons. On the other hand, classes carrying the whole X chromosome of race B (non-*bd*, non-*y*, non-*s*) may be compared with those having the right limb of the X of race A (classes showing *s*, compare 9 and 41, 10 and 42, 25 and 57 etc.). The comparison shows that in this case the substitution of the right limb invariably results in a marked increase of the testis size. The conclusion follows that the right limb, as well as the left limb, of the X chromosome carry genes concerned with sterility.

In order to compare the relative efficacy of the two limbs of the X chromosome, classes carrying the left limb of race A and the right limb of race B must be compared with those having the left limb of B and the right limb of race A. The classes to be compared should, of course, have identical autosomes (compare 35 and 46, 36 and 45, 37 and 44, 38 and 43, 39 and 42, 40 and 41). It can be seen that classes having the left limb of race A have larger testes, and hence the left limb is more important than the right.

An analysis of the third chromosome, which in this experiment is marked by two genes, *or* and *pr*, can be carried through along lines similar to those for the X chromosome (compare classes 1–8 with 17–24, 9–16 with 25–32, and then 17 with 18, 21 with 22, 25 with 26 etc.). Since the

effect of the third chromosome as a whole on testis size is much weaker than that of the X chromosome, the analysis of the effect of the former is more difficult. Nevertheless, the majority of the figures indicate that (a) both the part of the third chromosome carrying *or* and that carrying *pr* are concerned with testis size, and (b) that the part containing *pr* exerts a greater effect than that containing *or*.

TABLE 2

Length of the testis (in μ) in the offspring of the cross:
(bd y s Ba Race A ♀ × or Cy Race B ♂) F₁ ♀ × or Race B ♂

CLASS NO.	PHENOTYPE	M ± m	σ	LIMITS	n
1	*or Cy*	475.4 ± 4.3	60.7	244–661	200
2	*or*	468.8 ± 4.3	60.0	244–661	200
3	*Cy*	565.3 ± 3.9	55.2	313–748	200
4	*Ba or Cy*	375.0 ± 4.5	57.1	174–557	158
5	wild type	561.5 ± 4.1	59.0	296–730	204
6	*Ba or*	336.5 ± 4.7	60.9	157–609	168
7	*Ba Cy*	393.8 ± 5.7	67.0	191–644	138
8	*Ba*	372.2 ± 4.7	56.7	157–574	144
9	*bd y s Ba*	428.6 ± 9.2	61.6	278–592	45
10	*bd y s Ba Cy*	325.6 ± 11.7	61.8	139–470	28
11	*bd y s Ba or*	278.4 ± 8.9	53.2	139–435	36
12	*bd y s*	156.8 ± 5.9	55.0	70–313	86
13	*bd y s Ba or Cy*	211.4 ± 5.9	39.8	70–365	46
14	*bd y s Cy*	123.0 ± 3.7	39.0	35–244	111
15	*bd y s or*	109.8 ± 3.6	37.8	35–261	108
16	*bd y s or Cy*	81.4 ± 3.6	35.7	0–157	98
17	*s or Cy*	284.5 ± 7.9	55.9	139–505	52
18	*s or*	300.0 ± 6.9	58.8	122–487	73
19	*s Cy*	458.0 ± 10.4	60.0	296–644	33
20	*s Ba or Cy*	257.9 ± 15.5	67.7	104–418	19
21	*s*	468.8 ± 8.5	59.9	313–661	50
22	*s Ba or*	330.9 ± 13.8	66.5	122–487	23
23	*s Ba Cy*	324.0 ± 14.0	56.0	209–470	16
24	*s Ba*	426.3 ± 14.9	64.9	278–574	19
25	*bd y Ba*	488.4 ± 14.3	53.2	383–644	14
26	*bd y Ba Cy*	453.1 ± 22.1	79.7	244–626	13
27	*bd y Ba or*	313.2		313	2
28	*bd y*	258.6 ± 13.5	63.5	122–435	22
29	*bd y Ba or Cy*	208.8		139–365	8
30	*bd y Cy*	198.5 ± 11.3	37.4	157–313	11
31	*bd y or*	115.4 ± 12.6	48.7	52–313	15
32	*bd y or Cy*	119.2		70–278	8

Backcrosses to race B

Race A females carrying the genes beaded, yellow, short, Bare and purple (*bd y s Ba pr*) were crossed to race B males carrying orange and Curly (*or Cy*). In the F_1 generation females heterozygous for all these genes were selected, and backcrossed to race B males homozygous for orange. The experiment is, then, analogous to that described above, with the exception that since the male to which the hybrid females are backcrossed

belongs to race B, all the offspring appearing in the next generation will necessarily carry one complete set of B race (instead of A race as in the preceding experiment) autosomes, and some of them may carry B race chromosomes exclusively. Moreover, the gene purple does not manifest

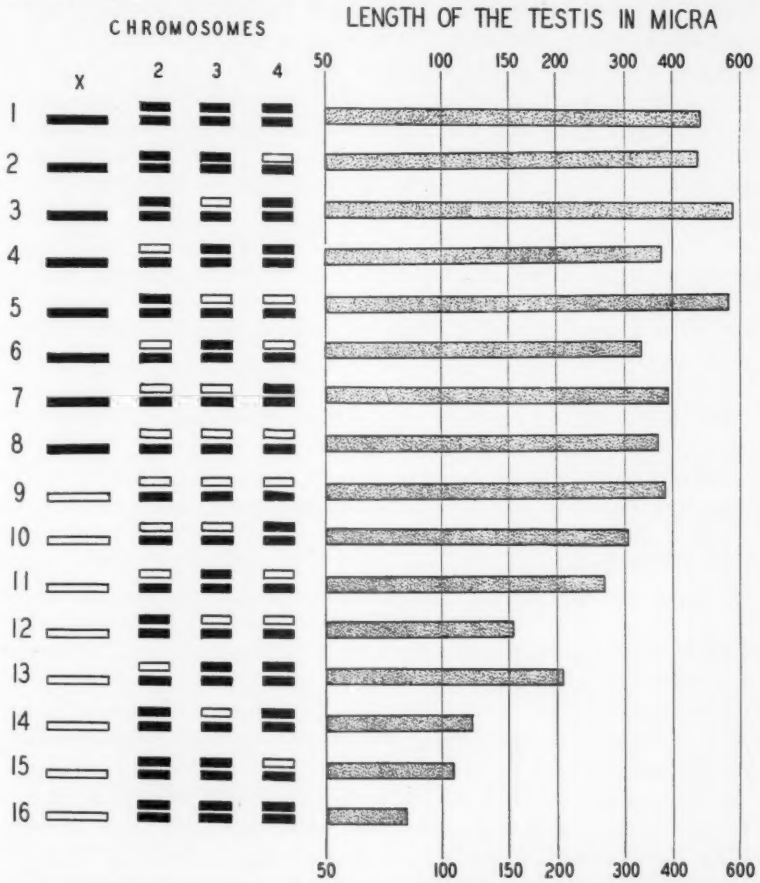

FIGURE 2. The chromosome constitution and the length of the testis in males appearing in the backcross of the F_1 hybrid females to race B males. Race A chromosomes—white; race B chromosomes—black. Only the non-crossover classes are represented. (The bar for class 9 should extend to 428.6; see table 2.)

itself in the offspring of the backcross, since the father of the backcross is homozygous for the wild type allelomorph of this gene.

The measurements of testis size in the males obtained from this backcross are summarized in table 2 and figure 2. Among the classes in which no crossovers are detected (classes 1–16), the first eight (1–8) carry the

X chromosome of race B, and, with one exception, have distinctly larger testes than the classes (9–16) carrying the X chromosome of race A. Thus, with a majority of the autosomes coming from race B, a race B X chromosome is required if large testes are to be formed—a result completely analogous, but reverse in sign, to that arrived at above by studying the backcross to race A males.

Among the males carrying the same X chromosome, the size of the testes depends upon the autosomes. Males receiving an X chromosome of race A (classes 9–16) have the largest testes if they carry one complete set of race A and one set of race B autosomes (class 9). If such males are made homozygous for race B fourth chromosome, the testis size is decreased (compare 9 and 10, 11 and 13, 12 and 14, 15 and 16). A similar, but stronger, effect is produced by homozygosis for the third chromosome of race B (compare 9 and 11, 10 and 13, 12 and 15, 14 and 16), and, finally, the second chromosome exerts the strongest effect (classes 9 and 12, 10 and 14, 11 and 15, 13 and 16). The conclusion is warranted that males having the race A X chromosome have the larger testes the more race A autosomes they carry.

By analogy with the conclusion just stated, as well as with the conclusion arrived at from the study of the backcross to race A, one might expect that in the present experiment males carrying race B X chromosome should have the larger testes the more race B autosomes they carry. Indeed, this is partly, but only partly, true. Among such males, those that are homozygous for race B fourth chromosome have larger testes than those carrying one race A fourth chromosome (compare classes 1 and 2, 3 and 5, 4 and 6, 7 and 8), and those homozygous for race B second chromosome have larger testes than those carrying one race A second chromosome (compare 1 and 4, 2 and 6, 3 and 7, 5 and 8). Turning to the third chromosome, one is at once struck by the fact that it shows a relationship that is the reverse of the expected one: males homozygous for the race B third chromosome have smaller testes than those possessing one third chromosome of race A (compare 1 and 3, 2 and 5, 4 and 7, 6 and 8). This inconsistency is to be discussed below.

Crossing over between *y* and *s* in the X chromosome produces males carrying an X the right limb of which (or a part thereof) comes from one race, and the left limb from the other race. One may observe that males having the right limb of the X chromosome from race A, and the left from race B (showing the effects of the gene *s*, but not of *bd* or *y*, in their phenotype), have mostly smaller testes than those carrying an intact X chromosome of race B (compare classes 17–24 with 1–8). Conversely, males carrying the right limb of the X from race B and the left from race A (showing *bd* and *y*, but not *s*), have larger testes than males with

complete race A X chromosome (compare 25–32 with 9–16). Finally, with the autosomes held constant, males having the right limb of the X from race A and the left limb from race B have mostly larger testes than males carrying the right limb of the X from B and the left from A (compare classes 17 and 32, 18 and 31, 19 and 30, etc.). It follows from this that (a) both limbs of the X chromosome carry sterility genes, and that (b) the sterility genes in the left limb are either more numerous or more effective than those in the right limb. Both conclusions are indentical with those reached from the study of the backcross to race A.

Returning again to the unexpected behavior of the third chromosome in males carrying the X chromosome of race B (see above), one might notice that in the experiment under consideration the cross was so arranged that all the hybrids originated from race A females (*bd y s Ba pr*), and, consequently, have race A cytoplasm. This fact may arouse the suspicion that homozygosis for the race B third chromosome is incongruent with race A cytoplasm, in such a way that in males carrying race A cytoplasm and all or a majority of race B chromosomes, the presence of one race A third chromosome may increase, instead of decrease, the testis size. If this were true, we would have the first indication that the cytoplasms of races A and B are specifically distinct and may be concerned with the production of the sterility of the hybrids. The possibility just stated can be subjected to a rigorous experimental test: a cross must be arranged which should produce offspring completely comparable with those from the cross just discussed in the chromosomal constitution, but which should have throughout the cytoplasm of race B, instead of that of race A. This requirement is fulfilled in the following experiment.

Race B females carrying *or* and *Cy* were crossed to *bd y s Ba pr* males of race A. F_1 females heterozygous for all these genes were backcrossed to race B males homozygous for *or*. All the strains used in this cross were the same as those used in the preceding experiments. The results are summarized in table 3. They should be compared to those presented in table 2. In these tables, the classes showing identical phenotypes have similar chromosomal constitutions, but differ in that the data of table 2 pertain to flies having race A cytoplasm, and those of table 3 to flies having race B cytoplasm. It is easily noticeable that the corresponding classes of males shown in table 2 have consistently larger testes than those shown in table 3. However, this fact is probably of no particular significance, since the experiments on which tables 2 and 3 are based were done at different times, and, due to the extreme sensitivity of the testis size to environmental conditions, no two separate experiments are likely to agree as far as the absolute size of the testes of hybrid males is concerned. Far more consequential is the relative testis size in males of different classes in each

TABLE 3

Length of the testis (in μ) in the offspring of the cross: (or Cy Race B ♀ × bd y s Ba Race A ♂) F_1 ♀ × or Race B

CLASS NO.	PHENOTYPE	M ± m	σ	LIMITS	n
1	*or Cy*	401.6 ± 7.2	72.9	226–609	103
2	*or*	404.9 ± 6.5	68.0	261–557	109
3	*Cy*	541.7 ± 7.4	80.6	313–731	118
4	*Ba or Cy*	298.6 ± 6.4	77.6	122–470	146
5	wild type	518.0 ± 6.6	71.9	348–679	120
6	*Ba or*	296.5 ± 6.3	72.4	139–487	133
7	*Ba Cy*	342.6 ± 7.7	82.7	157–522	114
8	*Ba*	359.1 ± 8.3	89.6	191–557	116
9	*bd y s Ba*	283.4 ± 10.3	63.2	174–452	38
10	*bd y s Ba Cy*	273.9 ± 9.6	64.6	174–400	45
11	*bd y s Ba or*	251.8 ± 7.8	62.1	139–383	63
12	*bd y s*	108.4 ± 3.2	41.9	35–261	171
13	*bd y s Ba or Cy*	194.5 ± 7.3	61.9	70–331	71
14	*by y s Cy*	79.9 ± 2.5	31.5	35–157	164
15	*bd y s or*	57.8 ± 2.6	31.5	17–139	149
16	*bd y s or Cy*	41.6 ± 2.5	32.7	0–139	177
17	*s or Cy*	213.7 ± 9.9	66.5	70–348	45
18	*s or*	206.4 ± 8.6	57.8	104–348	45
19	*s Cy*	330.1 ± 10.7	66.1	191–505	38
20	*s Ba or Cy*	179.7 ± 14.6	70.1	104–400	23
21	*s*	290.1 ± 11.8	67.7	174–417	35
22	*s Ba or*	195.4 ± 8.8	47.9	104–331	30
23	*s Ba Cy*	313.2		261–418	7
24	*s Ba*	252.3 ± 14.7	62.1	174–417	18
25	*bd y Ba*	464.1 ± 36.7	127.4	278–679	12
26	*bd y Ba Cy*	461.1 ± 22.5	74.1	278–557	11
27	*bd y Ba or*	438.0 ± 18.4	71.5	313–557	15
28	*bd y*	241.3 ± 10.4	67.9	122–418	43
29	*bd y Ba or Cy*	402.6 ± 13.3	44.0	331–4[illegible]7	11
30	*bd y Cy*	194.9 ± 8.9	56.4	87–365	40
31	*bd y or*	140.4 ± 7.7	49.9	52–261	42
32	*bd y or Cy*	122.0 ± 6.5	40.7	70–244	39
33	*y s*	643.8		626–661	2
34	*bd*	87.0		87	1
35	*bd Cy*	417.6		417.6	1
36	*y s or Cy*	556.8		556.8	1

experiment. Approaching the data from the standpoint of this criterion, one is forced to the conclusion that the data of tables 2 and 3 are identical in all essentials. For our purposes, the most important fact is that in males having race B cytoplasm and race B X chromosome, homozygosis for the race B third chromosome produces a decrease, instead of an increase, of the testis size. Hence, the anomalous behavior of the third chromosome in these crosses cannot be due to an interaction between this chromosome and the cytoplasm. No indication of the existence of an inherent difference between the cytoplasms of the two races is apparent.

Obviously, an explanation of the anomalous effect of the third chromosome in the crosses under consideration is to be looked for elsewhere. Two such explanations may be suggested. First, one may suppose that it is a specific property of the third chromosome of race A to increase the testis size in males having a majority of the chromosomes of race B. In this case, a male of hybrid ancestry having one race A third chromosome and the rest of the chromosomes of race B would always have larger testes than pure race B males. Second, the above behavior of the third chromosome may be due to a maternal effect, being manifested only in flies coming from the eggs of a hybrid mother. This amounts to assuming that the presence of the hybrid karyotype in the egg cell, before it has undergone the processes of maturation and fertilization, leaves an impression on this cell that lasts for at least one generation.

The two alternative explanations just suggested are obviously *ad hoc* hypotheses, and should be considered objectionable on this ground, were it not for the fact that they may be tested experimentally. Males of class 3 (table 3) have all the chromosomes of race B except a single race A third chromosome. Their race B third chromosome carries the marking gene *or*. Having large testes, these males are fertile, and can be crossed to pure B females homozygous for *or*. In the offspring of this cross two classes of males must appear. One of them, phenotypically orange, will have only race B chromosomes, and will be genotypically identical with males of classes 1 and 2 in table 3. The other class will be wild type in phenotype, and will be genotypically identical with class 3 in table 3, that is, will carry one race A third chromosome. Now, if the third chromosome of race A has *per se* the property of increasing testis size in males that are otherwise race B in constitution, the wild type males in this experiment should have larger testes than the orange males. Thus, the relationships observed in the first backcross generation (tables 2 and 3) should be repeated in the next generation. If, on the other hand, the phenomenon under consideration is due to a maternal effect, the testes of the wild type and the orange males should be either equal in size, or else the orange males should have larger testes than the wild type ones.

The experiment has been arranged as just outlined, and the testes in the resulting males were measured. Their size (in μ) was:

orange	607.6 ± 8.6
wild type	578.6 ± 7.4

The difference between these figures is not statistically significant, but the orange males have testes either equal to or larger than the wild type ones. The maternal effect hypothesis is correct. Maternal effects have been observed in *Drosophila pseudoobscura* crosses more than once (DOBZHANSKY 1935, DOBZHANSKY and STURTEVANT 1935).

STERILITY GENES IN THE SECOND CHROMOSOME

The experiments reported above show that genes responsible for the sterility of the interracial hybrids are located in the X, second, third, and fourth chromosomes—all chromosomes except the fifth, which has not been followed in the crosses. Furthermore, it has been shown that in the X and also in the third chromosome more than one sterility gene is present, located in different parts of the respective chromosomes. The question whether the second chromosome has one or more sterility genes could not be answered, since this chromosome carried a single gene marker, namely Bare. In the experiment now to be discussed this drawback is removed by introducing two marking genes in the second chromosome.

It may also be noticed that in the former experiments a majority of the marking genes were introduced through the race A parent, causing the classes of the offspring having mainly A race chromosomes to appear in relatively low frequencies. In the following experiment mainly race B markers are used.

Race B females carrying the sex-linked recessives scutelar (*sc*) and dela (se^d), and the second chromosome recessive cinnabar (*cn*) and dominant Smoky (*Sm*), were crossed to race A males carrying the fourth chromosome dominant Curly (*Cy*). The F_1 females heterozygous for these genes were backcrossed to race B cinnabar males. The results are summarized in table 4. The gene Curly involved in this cross was originally obtained as a mutant in race B, and "transferred" into race A by means of repeated backcrosses of Curly flies to race A males (see above). Thus, the race A fourth chromosome marked by *Cy* in this experiment is really a composite chromosome, containing, presumably, most of the material from race A, and a more or less small section including the locus of the gene *Cy* from race B.

To start with, one may notice that the data of table 4 corroborate the general conclusions regarding the action of the sterility genes previously discussed. Since males recorded in table 4 have at least one full set of race B autosomes, the classes carrying an intact race B X chromosome (1–4, 17–20) have larger testes than those carrying an intact X of race A (5–8, 21–24). Among males carrying the same X chromosome, largest testes are present in those that have most autosomes of the same race as the X chromosome (class 1—all chromosomes of race B, class 5—race A X chromosome and one set of A autosomes), and smallest testes in males having an X of one race and the autosomes of the other (class 4 with X of race B and one set of race A autosomes, class 8 with X of race A and race B autosomes). The fourth chromosome marked by *Cy* behaves as a race A chromosome in spite of the fact that it carries a section coming from race B. Crossing over in the X chromosome leads to the results expected on

the basis of the previously reported experiments: males having the left part of the X from race B (carrying *sc*) have larger testes than males having the right part of the X from race B but otherwise similar (males showing *se* but not *sc*).

TABLE 4

Length of the testis (in μ) in the offspring of the cross:
(sc se cn Sm Race B ♀ × Cy Race A ♂) F_1 ♀ × cn Race B ♂.

CLASS NO.	PHENOTYPE	M ± m	σ	LIMITS	n
1	*sc se cn Sm*	563.6 ± 6.7	56.6	418–679	103
2	*sc se cn Sm Cy*	523.2 ± 10.3	76.9	400–714	72
3	*sc se*	449.6 ± 10.6	84.4	278–626	61
4	*sc se Cy*	420.7 ± 9.3	70.1	261–592	56
5	*Cy*	319.5 ± 7.2	76.2	122–522	114
6	wild type	282.6 ± 6.1	67.0	122–435	122
7	*cn Sm Cy*	97.1 ± 4.0	36.9	35–174	85
8	*cn Sm*	90.7 ± 4.4	40.9	17–209	87
9	*sc cn Sm*	455.9 ± 10.0	58.3	365–592	34
10	*sc cn Sm Cy*	416.6 ± 12.5	70.5	226–592	32
11	*sc*	426.0 ± 10.0	74.1	261–592	55
12	*sc Cy*	435.4 ± 8.8	68.2	296–592	60
13	*se Cy*	433.3 ± 10.4	66.1	278–557	40
14	*se*	420.0 ± 11.7	78.0	278–592	45
15	*se cn Sm Cy*	120.6 ± 9.7	51.7	52–244	28
16	*se cn Sm*	86.0 ± 5.7	32.2	0–174	32
17	*sc se cn*	578.6 ± 9.2	63.3	452–661	48
18	*sc se Sm*	371.0 ± 9.7	60.9	209–487	39
19	*sc se cn Cy*	554.0 ± 12.6	78.7	388–696	39
20	*sc se Sm Cy*	364.2 ± 10.5	55.7	226–452	28
21	*Sm*	241.2 ± 9.9	74.1	87–452	56
22	*cn*	99.0 ± 4.5	33.1	52–243	54
23	*Sm Cy*	248.8 ± 9.0	69.4	104–418	60
24	*cn Cy*	121.3 ± 7.5	46.1	0–226	28
25	*sc cn*	492.9 ± 11.2	66.1	348–626	35
26	*sc Sm*	407.5 ± 11.8	60.4	313–557	26
27	*sc cn Cy*	500.6 ± 18.6	86.8	261–609	22
28	*sc Sm Cy*	396.5 ± 11.9	63.2	296–505	28
29	*se cn*	102.3 ± 7.8	39.8	52–191	26
30	*se Sm*	401.4 ± 12.8	61.6	226–487	23
31	*se cn Cy*	116.8 ± 8.8	43.0	70–261	28
32	*se Sm Cy*	463.9 ± 18.3	79.3	296–626	19

The detected crossing over in the second chromosome gives rise to males showing *Sm* but not *cn*, and *cn* but not *Sm* (classes 17–32). Males carrying the X and the second chromosomes of race A (classes 5 and 6) have larger testes than the corresponding classes of males in which a part of the second chromosome of race B is present (classes 21–24), but the latter have larger testes than males carrying the X of race A and the whole second chromosome of race B (classes 7–8). Males possessing the X and the second chromosomes of race B (classes 1 and 2) have testes equal to or larger than

similar males carrying a part of the second chromosome of race A (classes 17–20). In either case, the substitution of the right part of the second chromosome (carrying *Sm*) produces less effect than the substitution of the left part of the second chromosome (carrying *cn*).

The conclusion is warranted that more than one locus concerned with sterility is present in the second chromosome, and that the part of the chromosome marked by *cn* carries either more numerous or stronger sterility factors than the part marked by *Sm*. This result was not unexpected, in view of the fact that, according to the data of STURTEVANT, the gene *Sm* is easily transferred from race B to race A, whereas we have not been able to transfer *Ba* (lying very close to *cn*) from race A to race B. A less extensive series of attempts to transfer *cn* from race B to A was also unsuccessful.

Since the real position of the genes *cn*, *Ba*, and *Sm* in the second chromosome (in terms of the cytological map) is as yet unknown, it is not possible to decide which of the two parts of the chromosome is longer, and hence it remains obscure whether or not the effectiveness of a given chromosome section is proportional to its cytological length.

FERTILITY TESTS

The main body of our conclusions regarding the localization of the sterility genes is based on testes measurements, not on direct fertility tests. The justification of the procedure is given above, and need not be repeated here. Some fertility tests were performed as an additional check.

In the offspring of the backcross to race B the results of which are represented in table 2 males of the classes 1–8 were selected and crossed to pure race B females. Fifty males of each class were segregated into batches of five, and each batch was placed with 3–4 females in a separate culture bottle. No tests of individual males were made, since the males appearing in the backcrosses are, in contradistinction to males of pure races or the F_1 hybrids, rather weak, sometimes somatically abnormal, and in general inferior in vigor. The causation of this decrease in vigor in backcross males constitutes a separate problem (DOBZHANSKY and STURTEVANT 1935).

Males of the classes 5, 6, 7 and 8 produced no offspring. Hence, males carrying a second chromosome of race B and an X of race A are always sterile (which was to be expected since they have testes distinctly smaller than normal). All culture bottles containing males of the classes 1, 2, 3, and 4 produced offspring, although some of them did so only after repeated transfers on fresh food, and even then the offspring were few in number. The conclusion follows that males carrying all race B chromosomes in race A cytoplasm may be fertile, and the presence of the race A third or fourth chromosomes, or both together, with race B X and second chromosomes, does not necessarily prevent fertility.

THE SECOND BACKCROSS GENERATION

Females appearing in the first backcross generation fall into as many distinct genotypic classes as do their brothers. All of them possess a full set of the chromosomes of the race to which their father belonged, but the second set of the chromosomes inherited from the mother may be of either race, or may be partly derived from one race and partly from the other.

Some females that were sisters of the males whose testis measurements are presented in table 2 (coming from a backcross of the F_1 hybrid females to race B males) were individually crossed to race B males homozygous for orange. Since these females have their race A chromosomes marked by mutant genes, their own genetic constitution, as well as the genetic constitution of their male offspring, can be judged by the phenotype. No testis measurements were made on the males of the second backcross generation, but the testis size was evaluated by dissection and a simple inspection.

The results obtained are summarized below.

1. Females carrying all chromosomes of race B (*or Cy* in phenotype). Four such females tested; all sons have testes of normal size.

2. Females carrying an X of race A, rest of the chromosomes race B (*or Cy* in phenotype). One female tested; wild type sons have large testes, *bd y s* sons small to intermediate.

3. Females carrying a race A fourth chromosome, rest of the chromosomes race B (*or* in phenotype). Two tested; all sons have large testes.

4. Females carrying a race A third chromosome, rest of the chromosomes race B (*Cy* in phenotype). Five tested; four produced all sons with large testes, one gave sons with testis size varying from large to intermediate.

5. Females as above, but also one race A X chromosome (*Cy* in phenotype). Two tested; *bd y s* sons have small testes, wild type ones large testes.

6. Females carrying one third and one fourth chromosome of race A, the rest of the chromosomes of race B (wild type in phenotype). Seven tested; two produced sons with large testes, and five gave sons with testes varying from large to intermediate.

7. Females as above, but also one race A X chromosome (wild type in phenotype). One tested; *bd y s* sons had very small testes, non-*y* sons large to intermediate.

8. Females having one second and one fourth chromosomes of race A, the rest of race B (phenotype *Ba or*). One tested; *Ba* sons with very small testes, non-*Ba* sons with testes of intermediate size.

9. Females having one second and one third chromosomes of race A, the rest of race B (phenotype *Ba Cy*). Four tested; wild type and *Cy* sons had

testes of intermediate size, *Ba Cy* sons—small testes, *Ba* sons—very small testes.

10. Females having one set of autosomes of race A, the X's and the other set of autosomes of race B (phenotype *Ba*). One tested; wild type sons had intermediate or small testes, *Ba* sons very small testes.

These data, meager as they are, corroborate the conclusions regarding the distribution of the sterility factors in the chromosomes reached in the main body of the work. The failure to test a large number of the backcross females is due to the fact that they are on the whole much weaker than the F_1 hybrid females, and frequently produce no offspring.

DISCUSSION

The possibility that the sterility of the interracial hybrids in *Drosophila pseudoobscura* is due to an accumulation of structural differences between the chromosomes of the two races may be considered excluded. Some arguments against this possibility were presented in an earlier publication (DOBZHANSKY 1934). More recently TAN (1935a, b) has studied the chromosomes of the salivary gland cells in both races, and has found that they differ in six inverted sections, four of which are located in the X chromosome, one in the second and one in the third. Since in *D. pseudoobscura* the sterility is confined to the male hybrids, only the two autosomal inversions come under consideration as a possible cause of sterility. *D. melanogaster* individuals of either sex heterozygous for five inversions (delta-49 in the X chromosome, CIIL and CIIR of Curly in the second, CIIIL and CIIIR of Deformed in the third chromosome) are fertile. According to the unpublished data of STURTEVANT, the wild strains of *D. pseudoobscura* found in nature are frequently heterozygous for inverted sections in the third, and less frequently also in the second and in the X chromosomes, but no sterility is apparent in these strains. The autosomal interracial inversions discovered by TAN are remarkable neither in their cytological length nor in the extent of the suppression of crossing over they produce in the chromosomes concerned. The fourth chromosomes of the two races are, according to TAN, similar in gene alignment, but a failure of their pairing is frequently observed in the interracial hybrids. In short, the evidence against these inversions directly causing the sterility in *D. pseudoobscura* appears conclusive.

The data presented in this article show that genetic factors causing sterility of the interracial hybrids exist in all the chromosomes tested, that is in all the chromosomes except the fifth. Moreover, in the X, the second, and the third chromosomes respectively more than one sterility factor was detected. On the other hand, the effects of the different chromosomes are not equally strong: the left limb of the X has the strongest effect, the

right limb of the X, the second, the third, and the fourth chromosomes are decreasingly effective in the order indicated. This fact may be interpreted as indicating that "main sterility factors" lie in the X and in the second chromosomes, while the third and the fourth contain minor contributing factors or modifiers. This interpretation is, however, not a necessary one. The cytological lengths of the chromosomes (in salivary gland cells, according to TAN) decrease in the following order: X chromosome, second, third, and fourth chromosomes. Hence, the effectiveness of a chromosome in producing sterility is on the whole proportional to its length. That no strict proportionality of this sort obtains is clearly demonstrated by the greater effectiveness of the shorter left limb of the X as compared with the cytologically longer right limb of the same chromosome, but the available data permit no classification of the sterility factors into main and modifying ones. The greater effectiveness of the part of the second chromosome carrying the loci of Bare and cinnabar as compared with the part of the same chromosome carrying Smoky also gives no evidence on this point since the cytological lengths of these parts are as yet unknown. Likewise, the question of whether or not the parts of the chromosomes carrying interracial inversions are especially likely to contain numerous or powerful sterility genes remains open.

The available data are best interpreted as meaning that the testis size in the backcross hybrids is the larger the more their X chromosome agrees with the autosomes as to its racial origin. In other words, sterility versus fertility seems to be determined by interactions of factors located in the X chromosome with factors located in the autosomes. Here again, this interpretation is not a necessary one, since the cytological length of the X of *D. pseudoobscura* is almost equal to the sum of the lengths of all the autosomes. Hence, the data are not inconsistent with the supposition that testes are smallest in males carrying equal volumes of the chromosomal material from both races, and that the more homogeneous are the chromosomes in their racial origin, the larger are the testes.

Concerning the mechanism of the action of the sterility factors, the available data permit a single conclusion only, namely that their effects on testis size are additive. This is amply demonstrated by the figures in tables 1–4: if two chromosomes, or sections of chromosomes, each produce a decrease (or an increase) in testis size, these two chromosomes, or sections, produce a larger decrease (or increase) if they are present simultaneously. More complicated forms of interactions, for instance factors whose effects are contingent on the presence of other factors, have not been detected. The only exception is the third chromosome of race B, the action of which in the hybrids possessing predominantly race B chromosomes (or at least the race B X chromosome) is complicated by a maternal effect

(see the discussion of this point in the text). It is of interest that in hybrids carrying predominantly race A chromosomes the third chromosome does not show any anomalous effects.

SUMMARY

1. Crosses between race A and race B of *Drosophila pseudoobscura* produce in F_1 fertile daughters and sterile sons. The F_1 females may be backcrossed to males of either parental race. Some of the males in the resulting offspring are fertile and others are sterile. Fertile males always have large testes, while testes in the sterile males vary in size from normal to very small. The testis size is an index of the degree of disturbance in the process of spermatogenesis, the disturbance being greatest in the smallest testes.

2. Backcross males having only race A chromosomes are fertile irrespective of whether they have the cytoplasm derived from race A or from race B. Backcross males having only race B chromosomes are likewise fertile irrespective of the source of their cytoplasm.

3. In the offspring of the backcross of the F_1 hybrid females to race A males, the males carrying the X chromosome from race A have larger testes than those carrying the X chromosome from race B (table 1, figure 1). Among classes carrying the same X chromosome testis size depends upon the autosomes: the more the autosomes agree in their racial origin with the X chromosome the larger are the testes, and vice versa.

4. Backcrosses of the F_1 hybrid females to race B males give results analogous to the above but reverse in sign: the largest testes are present in backcross males having the race B X chromosome and race B autosomes, and smallest testes in males carrying race A X chromosome and B race autosomes (tables 2, 3, and 4, figure 2).

5. All the chromosomes studied carry genes concerned with testis size, and consequently with sterility. The X chromosome produces, however, the strongest effect, the second chromosome follows next, and the third and the fourth chromosomes last. Thus, on the whole, the effectiveness of each of the chromosomes is proportional to its cytological length.

6. Wherever in our experiments a chromosome was marked by more than one mutant gene it was possible to show that sterility genes are present in different parts of this chromosome.

7. The effects of the sterility genes located in the different chromosomes and sections of the chromosomes are additive.

8. The behavior of the third chromosome of race B in the hybrid males of the first backcross generation possessing a majority of race B chromosomes is anomalous. Such males have larger testes if they carry one race A and one race B third chromosome than if they are homozygous for the race B third chromosome. This anomaly is due to a maternal effect: in in-

dividuals coming from the eggs of the F_1 hybrid females homozygosis for the race B third chromosome decreases the testis size.

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SEX REVERSAL IN APLOCHEILUS LATIPES AND A NEW EXPLANATION OF SEX DIFFERENTIATION

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INTRODUCTION

IN *Aplocheilus latipes* where the male is digametic XY two allelomorphic genes concerning the body color, orange-red (*R*) and white (*r*) are located in the X and Y chromosomes (AIDA 1921). Heterozygous orange-red males X*R*Y*r* in crosses with white females X*r*X*r* produce orange-red females X*R*X*r* and white males X*r*Y*r* in criss-cross manner, though sometimes a few exceptional orange-red males may be found. Some of these abnormal males produce in crosses with normal females offspring with an abnormal sex ratio, the females always predominating in number and the males which are all abnormal like their male parent being very few or not present at all. By carrying on the breeding of these males generation after generation I found that the proportion of males gradually increases (AIDA 1930), and I tried in the former report to explain these abnormal males as the product of non-disjunction in sex chromosomes having XXY constitution.

WINGE (1930) found in *Lebistes reticulatus* sex-reversed males of female genotype XX which in crosses with normal females produced female offspring only. On account of the similarity of breeding results between his observation on sex reversal and ours on abnormal males he suggested that our male fish are also produced by sex reversal, but not by non-disjunction.

To determine which one of these alternative conceptions is more valid further research has been continued. A new fact irreconcilable with the supposition of non-disjunction was found, and at present I am inclined to believe that our males are, as WINGE has assumed, sex-reversed males of the female genotype XX. Moreover I was able to produce at the same time females which are unquestionably due to sex reversal, viz. females of male genotype XY.

The possibility of sex alteration in so well differentiated a gonochorist as Aplocheilus and the breeding results induced me to make a new hypothesis on sex differentiation in our fish.

SEX-REVERSED MALE OF FEMALE GENOTYPE

1. *Effect of individuality of male parent on sex differentiation*

Among a great number of matings of abnormal males in 1927 (table 4, AIDA 1930) the male designated as K10,8 produced male offspring in the highest ratio, viz. 4 ♀ :1 ♂, and male M2 also in pretty high ratio, viz. 10 ♀ :1 ♂. In 1928 some male offspring from these two matings were each mated to five females from the same litter as the male.

TABLE 1

Male offspring of the males K10, 8 and M2 crossed each with 5 females from the same litter as the male. R = Orange-red. r = White.

NO. AND YEAR OF MATING	PARENTS		OFFSPRING				TOTAL		RATIO
	♂	♀	♀♀R	♀♀r	♂♂R	♂♂r	♀♀	♂♂	♀:♂
B.26 '28	R K10, 8, 3	R 5	190	71	27	15	261	42	6
B.27 '28	R K10, 8, 2	R 5	145	63	27	27	213	54	4
B.28 '28	R K10, 8, 4	R 5	149	44	6	1	193	7	28
B.30 '28	R K10, 8, 5	R 5	61	27	3	0	88	3	29
B.31 '28	R K10, 8, 6	r 5	104	83	8	5	187	13	14
B.32 '28	R K10, 8, 7	r 5	92	78	8	4	170	12	14
B.16 '28	R M2, 4	r 5	47	17	3	1	64	4	16
B.17 '28	R M2, 6	r 5	291	284	50	46	575	96	6
B.18 '28	R M2, 2	r 5	37	53	5	5	90	10	9
B.19 '28	R M2, 8	r 5	324	343	62	68	667	130	5
B.20 '28	R M2, 7	r 5	80	79	9	7	159	16	10

In the above tabulated matings the ratio of males to females varies from 4 to 29, and no constancy is found notwithstanding the fact that the parents of each mating were taken from the same litter.

All matings were carried on at the same season of the year under similar conditions as possible, so that the difference of external influences is excluded. As in each mating the five females, all taken from the same litter, were crossed with the single male, if some different tendency in producing the male number should exist in the female parent, it might have been mitigated, and would not affect so much the ratio of male number in offspring as we have seen. The actual variation of the ratio, accordingly, may be considered to depend on the nature of the male parent.

2. *Effect of individuality of female parent on sex differentiation*

In the next year (1929) a single male was mated simultaneously to two females in the same aquarium, each from different litters, and the sex numbers were counted in the offspring of each female parent separately.

TABLE 2

Three orange-red males, A, B, and C, from mating 26, 1928, were each mated to single orange-red female from the same litter and also simultaneously to single white female from mating 29, 1928, where only females and no males were produced 170 ♀♀, 0♂.

R = Orange-red. r = White.

NO. AND YEAR OF MATING	PARENTS ♂	PARENTS ♀	OFFSPRING ♀♀R	OFFSPRING ♀♀r	OFFSPRING ♂♂R	OFFSPRING ♂♂r	TOTAL ♀♀	TOTAL ♂♂	RATIO ♀:♂
B.9 '29	A	*R* from B.26, '28	62	24	33	12	86	45	2
B.12 '29	A	*r* from B.29, '28	38	30	31	29	68	60	1
B.10 '29	B	*R* from B.26, '28	97		21		97	21	5
B.13 '29	B	*r* from B.29, '28	136	1			137	0	No males
B.11 '29	C	*R* from B.26, '28	77	18	33	13	95	46	2
B.14 '29	C	*r* from B.29, '28	69	59	6	1	128	7	18

The breeding results in the table 2 show clearly that the same male may produce in matings to different females male offspring in different ratios. That this variation of the ratio is caused by the difference of the individual tendencies of female parents is to be conjectured.

When the females are taken from the wild stock and bred to any abnormal male, the offspring are always all female with none or very few males. Even the males which in crosses with colored females produce many male offspring yield in simultaneous crosses with wild females female offspring

only. The breeding results of the same male mated to the colored and wild females at the same time in the same aquarium are summarized in the next table.

TABLE 3

Abnormal male crossed with colored and wild females.

BR=Brown, the wild color. *B'R*=Orange-red variegated with black. *B'r*=White variegated with black. *R*=Orange-red. *r*=White.

NO. AND YEAR OF MATING	PARENTS ♂	PARENTS ♀	OFFSPRING ♀♀	OFFSPRING ♂♂	TOTAL ♀♀	TOTAL ♂♂	RATIO ♀:♂
B.25 '32	*B'r* (B.20,'31)	*R*	*B'R* 16 *B'r* 16 *R* 7 *r* 10	*B'R* 16 *B'r* 17 *R* 12 *r* 13	49	58	1
B.34 '32		*BR* (Wild)	*BR* 120	0	120	0	
B.1 '33	*R* (B.28,'32)	*r* (B.28,'32)	*R* 74 *r* 81	*R* 19 *r* 22	155	41	4
B.2 '33		*BR* (Wild)	*BR* 153	*BR* 0	153	0	
B.15 '33	*B'R* (B.26,'32)	*r* (B.26,'32)	*B'R* 29 *B'r* 29 *R* 23 *r* 22	*B'R* 0 *B'r* 3 *R* 6 *r* 3	103	12	9
B.16 '33		*BR* (Wild)	*BR* 310	0	310	0	

Table 3 shows that the very same males which have produced in crosses with colored females males in the ratio of one-fifth, one-tenth or even more than one-half of the total offspring, yielded in mating to wild females, females only and no males at all. The wild females, nevertheless, do not produce constantly females only, thus sometimes when the number of the offspring reared is large enough a few males may be found. For instance, in mating 2, 1931, where an abnormal male was mated to three wild females, only one male was found among 719 offspring, the remaining ones being females.

From these results the inference may be drawn that the females of our colored varieties have some tendency to produce a greater number of male offspring than the wild normal females.

3. *Seasonal influence on sex differentiation*

The ratio of males produced varies according to the different seasons. Our fish spawn every morning throughout the whole warm season, and

the sex ratio in the offspring from the same parents differs according as they are hatched out in early or mid-summer. In the next table the numbers of male and female offspring from the same parents reared in each period are separately listed. The water temperature at the surface in the morning before sunrise was in the first period 23–26°C. and in the second 25–28°C.

TABLE 4

Offspring of the same parents hatched out in earlier and later periods of summer.

BR, *B'R*, *B'r*, *R'r* same as in former table. *Br* = Blue.

NO. AND YEAR OF MATING	PARENTS ♂	PARENTS ♀	HATCHED OUT PERIODS	OFFSPRING ♀♀				OFFSPRING ♂♂				TOTAL ♀♀	TOTAL ♂♂	RATIO ♀:♂
B.6 '29	*R*	*R* (B.31,'28)	From June 26 to July 20	*R*	54	*r*	20	*R*	0	*r*	1	74	1	70
			From July 21 to August 10	*R*	52	*r*	21	*R*	18	*r*	7	73	25	3
B.7 '29	*R*	*R* (B.31,'28)	From June 26 to July 20	*R*	38	*r*	15	*R*	9	*r*	2	53	11	5
			From July 21 to August 16	*R*	16	*r*	6	*R*	17	*r*	7	22	24	1
B.8 '29	*R*	*R* (B.31,'28)	From June 26 to July 23	*R*	53	*r*	24	*R*	4	*r*	1	82	5	16
			From July 24 to August 17	*R*	40	*r*	20	*R*	30	*r*	10	60	40	1.5
B.23 '32	*BR*	*r* (B.2, '31) From normal stock	From July 4 to July 20	*BR*	11	*Br*	9	*BR*	9	*Br*	8			
				R	10	*r*	9	*R*	1	*r*	6	39	24	2
			From July 25 to August 6	*Br*	11	*Br*	11	*BR*	1	*Br*	4			
				R	17	*r*	12	*R*	1	*r*	3	51	9	6
B.27 '32	*B'R*	*r* (B.16,'31)	From July 10 to July 20	*B'R*	9	*B'r*	9	*B'R*	2	*B'r*	1			
				R	7	*r*	5	*R*	0	*r*	0	30	3	10
			From July 24 to August 6	*B'R*	24	*B'r*	10	*B'R*	8	*B'r*	9			
				R	26	*r*	11	*R*	5	*r*	8	71	30	2

From the table we can see clearly that the season has a certain influence in determining the ratio of male offspring. In four matings among five the increase of the male number is very large in the second period. Probably high temperature may induce some females to male reversal. WINGE (1934) reports similar seasonal influence on the sex ratio in offspring of sex-reversed males of Lebistes. Thus he found that though in the early summer a relatively large number of males is produced, in other seasons the number of females increases, the females alone being produced in

winter. According to WITSCHI (1929) female tadpoles of *Rana sylvatica* reverse to males at high temperature. Thus the temperature seems to have a similar effect on sex differentiation in lower vertebrates.

4. *Excess of male number*

The mating 9, 1930, yielded male offspring in a high ratio, viz., 140 ♀ ♀ and 81 ♂ ♂. In the next year 10 ♂ ♂ and 19 ♀ ♀ from them were bred in mass (B.20, '31). The result was 105 ♀ ♀ and 82 ♂ ♂; the male ratio increased a little over that of the parents' brood. One male among them, orange-red variegated with black, was paired with a normal white female. This mating produced 143 ♂ ♂ and 45 ♀ ♀; so the male number amounts to more than thrice that of the female, an enormously high male ratio. The details are listed in table 5.

TABLE 5

Mating 26, 1932: B′R male crossed with r female.
B′R, B′r, R, r same as in former tables.

SPAWNING INTERVAL	NUMBER OF FRY ABOUT A MONTH OLD	♀♀				♂♂				TOTAL		RATIO
										♀♀	♂♂	♀ : ♂
From July 9	98	*B′R*	12	*R*	4	*B′R*	16	*R*	16			1
to July 20		*B′r*	4	*r*	3	*B′r*	20	*r*	22	23	74	3
From July 24	72	*B′R*	4	*R*	2	*B′R*	10	*R*	6			1
to August 6		*B′r*	6	*r*	6	*B′r*	6	*r*	12	18	34	2
From August 14	210	*B′R*	0	*R*	2	*B′R*	9	*R*	9			1
to August 29		*B′r*	0	*r*	2	*B′r*	15	*r*	2	4	35	9
								Grand total		45♀♀	143♂♂	

The discrimination of sex in our fish according to external aspects which is not possible in young stages may be made only after maturity, when the male manifests its secondary sexual characters, viz., long dorsal fin and wide anal fin with minute horny processes on fin rays (OKA 1931) and when the female lays eggs. From hatching to maturity there is an interval of about one year, so that the young individuals must pass the cold season, many weak ones dying during this hibernation.

Some suspicion might be raised concerning the sex ratios above in that through some unknown cause the majority of the female offspring might die to cause an unusually high male ratio. In the offspring of the third period the rate of mortality is great, thus only 39 adult fishes were reared from 210 fry. But in those of the first period nearly all of the fry became fully grown, and the disturbance of the sex ratio due to death is not observable. The preponderance of the male offspring in this case, therefore, must be due to the special nature of the parents, and this fact alone suffices

to deny my former supposition of non-disjunction in the origin of abnormal males. If the latter were the products of non-disjunction and had XXY chromosomes, it should be quite impossible to produce more male offspring than female. In our experiment the male number amounts to more than thrice that of the female. It is clear that our former supposition of non-disjunction must be abandoned, and the hypothesis of sex-reversal must be adopted instead of it.

From all these breeding results we can see that the sex ratio of the offspring of abnormal males crossed with any female of colored varieties varies according to the individuality of the two parents and it differs even in different parents taken from the same litter. Through the continual selection of the abnormal male as parent from the litter where the male ratio is high the male offspring may gradually increase until eventually they are in excess. The increase in the proportion of male offspring generation after generation, that I reported in my last paper (1930) is also probably the result of selection, as I have always chosen the male as parent of the next mating from the litter in which a relatively large number of males was found.

SEX-REVERSED FEMALE OF MALE GENOTYPE

A mass mating of normal strain, 1929, where ten white females (X*r*X*r*) were crossed with five orange-red males (X*R*Y*r*) gave the normal offspring composed of 614 orange-red females, 423 white males and a single exceptional white female. The last one was paired with a single normal orange-red male (X*R*Y*r*), the result of which was quite remarkable, as shown in the next table.

TABLE 5

Exceptional white female crossed with single normal orange-red male (XRYr).
Mating 41, 1930

	ORANGE-RED	WHITE	TOTAL
♀♀	55	0	55
♂♂	55	112	167

If the exceptional female were of normal constitution X*r*X*r* the orange-red and white offspring should be female and male respectively. We see however that half of the orange-red offspring are males: thus among 110 orange-red individuals one-half, i.e., 55 are male and another half female; while all white individuals, 112 in all, are male. In total the number of the male offspring is thrice that of female.

This singular result may be well explained by supposing that the exceptional female was produced by sex reversal and has X and Y chromosomes. Such a white female X*r*Y*r* mated to a normal orange-red male

X*RYr* will produce offspring composed of orange-red females X*RXr* and males X*RYr*, as well as white males X*r*Y*r* and Y*r*Y*r* in equal ratio, so that the male number will be thrice that of female if the YY males survive.

If this supposition is correct, half of the white male offspring must have two Y chromosomes and in mating with normal female XX produce male offspring only. To test this supposition, three white males were taken at random and each was mated to three normal females respectively. The results are listed in the next table.

TABLE 6

3 white males from B.41, 1930, crossed each with 3 normal orange-red females.
R, r = same as in former tables.

NUMBER AND YEAR OF MATING	MALE PARENT	OFFSPRING ♀♀		OFFSPRING ♂♂		TOTAL	
B.17, 1931	A	*R*	14	*R*	8	♀♀	25
		r	11	*r*	15	♂♂	23
B.18, 1931	B	*R*	1	*R*	5	♀♀	6
		r	5	*r*	4	♂♂	9
B.19, 1931	C	*R*	0	*R*	13	♀♀	0
		r	0	*r*	9	♂♂	22

Though since the number of offspring reared from each mating above listed is small the results cannot perhaps be regarded as quite conclusive, yet they are in favor of the supposition just stated. Two males, A and B, produced offspring with a normal sex ratio, while C male produced only males. Ten offspring from the C male, i.e., five orange-reds and five whites were tested by pairing to normal white and orange-red females respectively. The results were quite normal, because the former yielded offspring consisting of orange-red females and white males in criss-cross manner, and the latter both orange-red and white females and males in equal ratio. From these facts it may be inferred that all male offspring of C male are of the normal XY constitution and consequently the C male must have two Y chromosomes.

The orange-red males of the same litter as C male (B.41 '30) were all normal. Three fishes among them were crossed with three normal white females respectively. The results showed normal criss-cross inheritance: all orange-red offspring were females and all whites were males, as shown in table 7.

The single exceptional white female in mating 11, 1931, is the second fish produced by sex reversal which I obtained, and breeding experiments similar to the above stated were repeated with this female: it was paired with a single orange-red male from normal stock. The result was quite similar to that of B.41, '30 (table 8).

TABLE 7

Offspring of 3 orange-red males from B.41, '30, crossed each with 3 normal white females.

NO. AND YEAR OF MATING	ORANGE-RED		WHITE	
	♀♀	♂♂	♀♀	♂♂
B.9, '31	219	0	0	204
B.10, '31	154	0	0	182
B.11, '31	105	0	1	95

TABLE 8

Exceptional white female from B.11, '31, paired with a normal orange-red male. Mating 22, 1932.

	ORANGE-RED	WHITE	TOTAL
♀♀	217	0	217
♂♂	222	374	596

The mode of color inheritance was quite normal, orange-red and white offspring being nearly in equal ratio, as the male parent was heterozygous for its color. One half of the orange-reds were males and the other half females. The total number of males was thrice that of the females as in B.41, '30.

Under the expectation that one half of the white males would yield male offspring only, ten individuals among them were paired each with a single female. The results are shown in table 9.

TABLE 9

Offspring of 10 white males from B.22, '32, crossed with orange-red females.
R = Orange-red. *r* = White.

NO. AND YEAR OF MATING	FEMALE PARENT	NUMBER OF FRY ABOUT A MONTH OLD		ADULT OFFSPRING				TOTAL	
	R	*R*	*r*	*R*♀♀	*R*♂♂	*r*♀♀	*r*♂♂	♀♀	♂♂
B.5, '33	from same litter as male	274	263	32	30	28	31	60	61
B.6, '33	"	173	168	0	119	0	116	0	235
B.7, '33	"	84	102	27	23	35	18	62	41
B.8, '33	"	126	142	0	116	1	116	1	232
B.9, '33	"	202	199	85	68	68	75	153	143
	R								
B.10, '33	from normal stock	254	239	94	104	93	90	187	194
B.11, '33	"	195	219	0	118	0	131	0	249
B.12, '33	"	256	264	101	83	88	93	189	176
B.13, '33	"	306	342	69	76	61	66	130	142
B.14, '33	"	93	86	1	63	0	59	1	122

In four matings B.6, B.8, B.11 and B.14 among ten, only male offspring were produced, and in two matings B.8 and B.14 one single exceptional

female was found. The remaining six matings yielded offspring of normal sex ratio, i.e., the female and male nearly in equal proportion. The death rate of young fishes during hibernation is not small, as already stated, and is especially heavy when they are attacked by epidemic disease as in B.5, but in those four matings the greater number of fry was reared up to maturity and there is scarcely any doubt that all females in them were killed and the males alone survived. It is plausible to think that the male parents in those matings carry two Y and no X chromosomes at all.

The fact that four males among ten, i.e., one-half of the whole male offspring carry two Y chromosomes well verifies the supposition that the mother fish of these males, the exceptional white female, is the sex-reversed female of male genotype XY.

For the test of the brother orange-red males (B.22 '32) ten fishes among them were mated to normal white females or orange-red females from the same litter as males. Two matings were unfruitful, the other eight all showed normal inheritance so that all orange-red males of B.22 '32 are to be supposed to have the normal constitution of XY (table 10).

TABLE 10

Orange-red males from B.22, '32 mated to orange-red females of the same litter or to white females from normal stock.

R, *r* same as in former table.

NO. AND YEAR OF MATING	FEMALE PARENT	NUMBER OF FRY ABOUT A MONTH OLD		ADULT OFFSPRING			
	r	*R*	*r*	*R*♀♀	*R*♂♂	*r*♀♀	*r*♂♂
B.18, '33	from normal stock	266	217	247	2	0	197
B.20, '33	"	145	146	138	0	0	135
B.21, '33	"	263	271	187	1	0	179
	R						
B.22, '33	from B.22, '32	297	95	145	67	0	63
B.23, '33	"	442	138	194	100	0	84
B.24, '33	"	454	148	239	114	0	116
B.25, '33	"	216	72	30	22	0	17
B.26, '33	"	252	69	178	63	0	62

Quite similar results were obtained by WINGE (1934) on the sex-reversed female of *Lebistes reticulatus*, which produced in a cross with normal male 21 females and 81 males, i.e., in the ratio of 1:3. One-third of the males were reported to be YY males, and in crosses with normal females produced male offspring only, just as in our case.

It is very interesting to see that in fishes which are well sex-differentiated like Lebistes and Aplocheilus one sex may change to the opposite one, and that YY male may be viable and fertile while it is lethal in Drosophila.

DISCUSSION AND A NEW SUPPOSITION ON SEX DIFFERENTIATION

At present the elucidation of sex differentiation is based on the fundamental conception, proposed by Morgan, of two antagonistic and competing female and male determining factors. Generally it is understood that when the female factor *F* predominates the individual becomes female, while when the male factor *M* prevails it becomes male. But about the locations of these factors in the germ cells the opinions of different authors do not agree.

GOLDSCHMIDT (1934) in his explanation of intersexes in Lymantria (female digamety) locates the *M* factor in X chromosome and the *F* factor in the cytoplasm of the ovum, *FMM* being male and *FM* female. KOSSWIG (1931) to explain sex differentiation in the hybrid between *Platypoecilus maculatus* (female digamety) and *Xiphophorus Helleri*, supposes in the former the *M* factor to be located in autosomes and the *F* in W or Y chromosome; the Z or X chromosome being quite indifferent in sex determination has none of *F* or *M* factor. According to BRIDGES (1925) in Drosophila (male digamety) all autosomes have both female and male genes, and in total the male genes are more effective than the female. The X chromosome in contrast to autosomes contains net female genes, and the sex is differentiated according to the ratio of these two sets of genes. WITSCHI (1929) assumes in European frog (male digamety) that the autosomes have net *M* factor and X chromosome net *F* factor as in Drosophila, and moreover Y chromosome carries a variable *f* factor, the allelomorph of *F* and lower in its strength than the single *M*. WINGE (1934) in the interpretation of sex reversal in Lebistes (male digamety) supposes that the net factor of each autosome is different in quality and strength, some being feminine, and others masculine in various strengths. The X chromosome is feminine, while the Y chromosome is masculine and stronger than a single X. The sex reversal is considered to be caused by the accumulation of autosomes of the same sort in an ovum, whose factors being all feminine or masculine surpass in total the antagonistic effect of sex chromosomes so that an individual XX may be changed to male and the XY to female.

Such diversities of the opinions of different authors about the location of *F* and *M* factors induced me to doubt their real existence. From the facts that in Aplocheilus as well as in Lebistes females of male genotype and males of female genotype may be produced through sex reversal, and that the males of female genotype produce in crosses with normal females offspring with sex ratios varying from all females to a preponderance of males, I am induced to make the following hypothesis on sex differentiation.

The genes which correspond to the primary sexual characters of both sexes are distributed in autosomes, and they become activated by certain

definite genes which act as stimulating genes. We may think that the sex differentiation is due to the difference of the quantity of such stimulating genes. When it is greater than a certain limiting value the feminine genes only are activated and the action of masculine genes is suppressed so that the female characters will develop, while on the contrary when it is smaller just the reverse will take place.

The genes concerned in stimulating, which we may call sexual exciters, are located in the sex chromosomes. In the heterogametic male the total sum of sexual exciters in X and Y chromosomes is less than that contained in the two X chromosomes, and in the heterogametic female their total quantity in X and Y or Z and W chromosomes is greater than that in two X or Z chromosomes; in other words, in the former case the X chromosome has greater exciting quantity or potency than the Y and in the latter case we have the reverse. As thus the differentiation of sexes is considered to be caused by the difference of quantity of the same exciting agency there should be a threshold value between the two sex determining quantities and when either of the latter is greater than that value the action of female genes and in the contrary case that of male genes will be stimulated.

Though some external or internal conditions might influence these exciting potencies of sex chromosomes, in normal case their disturbing action is not so great as to cause the total sum of the potencies of sex chromosomes to pass over the threshold value and produce abnormal sex differentiation.

According to my opinion the sex reversal in our fish may be explained by an unusual disturbance of the potency of the X chromosome. In our breeds of colored varieties, I think, the X chromosome loses the constancy of its potency by some unknown cause, and so fluctuates always in varying range that in some fishes which happened to have lower fluctuants of the X chromosome the total sum of their potencies falls under the threshold value, so that the masculine genes only are activated notwithstanding their female genotype, and the sex is reversed.

The range of fluctuation is supposed not to be equal in all X chromosomes and it varies even in those of offspring produced from the same parents. The variation of the number of sex-reversed males due to the individuality of both parents in the offspring of the XX male crossed with the female of colored varieties will be the result of the varying ranges of fluctuation in the X chromosomes of the latter. The wider the range of their fluctuation in both parents, the greater number of males will be produced.

From the fact that in crosses with wild females any sex-reversed male yields generally female offspring only, rarely together with a few males, we may infer that the range of fluctuation is not very wide in negative direction, hence most of the lower fluctuants, excepting the lowest ones, in combination with a normal X chromosome of the wild species are unable to

depress the total sum of potencies so much as to let it pass over the threshold value and effect sex reversal.

The fluctuation of the X chromosome in our breed, in my view, would have taken place at first in one of two X chromosomes, and on account of many years' close inbreeding all colored varieties in our breeds came to get two fluctuating X chromosomes, whereupon some females which happened to carry two lower X chromosomes were first detected as the sex-reversed ones. By constant breeding of the sex-reversed males selected out from the litters where their number is high, the fluctuation of X chromosome may be shifted further in the negative direction, so that the number of the male offspring will be increased. The matings in table 5 are the results of such processes, where the excess of males may be found.

Probably the fluctuation of the X chromosome might be influenced easily by outer conditions, and the difference of the rate of male production from the same parents in different seasons is to be explained as the effect of different climate.

The fluctuation takes place in both directions either positive or negative, starting from normal value. The effect of the positive fluctuation cannot be recognized in the offspring of sex-reversed males crossed with normal females as then both parents carry X chromosomes only, and the increase in the potency of that chromosome results in nothing but to produce the females which are not different at all from the normal ones.

The sex-reversed female of male genotype XY is to be explained as the effect of the increase in the total potency of X and Y chromosomes. When the total sum of potencies increases and passes over the threshold value to the female side, a female will be produced in spite of the male genotype. Whether this increase of potency is caused by the fluctuation occurring in the X chromosome only or in both X and Y is not yet fully decided. But even through the consideration of the fluctuation in the X chromosome only the fact is easily understood. The single X chromosome of extreme fluctuation on the positive side, which will very rarely occur, accompanied by a Y chromosome, may have sufficient total potency to excite the female genes only. The rarity of sex-reversed females favors this supposition.

The experimental results of the sex reversal phenomenon in our fish are thus fairly explainable by the supposition that sex differentiation is due to differences in the total quantities of exciting factors in the sex chromosomes, and the fluctuation of the potency of the X chromosome.

Now to test how far this supposition of sex differentiation is conformable to the other facts about sex differentiation, I will try in the following to interpret some well known complex facts based on this supposition.

The most perplexing facts are those of Goldschmidt's intersexes and sex reversal in the hybrids between the different local races of *Lymantria dispar*. The results of the crosses designated as "Basic" by him and the

crosses between the very weak and strong races (GOLDSCHMIDT 1934) can easily be interpreted on the basis of our hypothesis with an auxiliary one that the maternal cytoplasm influences slightly the potencies of sex chromosomes: the cytoplasm of the strong race strengthens and that of the weak weakens the potencies of sex chromosomes.

To simplify the explanation we may assume schematically some numbers for the potencies of sex chromosomes and the threshold value in different races:

Japanese or strong race..... X20 Y40=60, X20 X20=40, the threshold value=52.
European or weak race ..... X25 Y37=62, X25 X25=50, the threshold value=55.
Very weak race..... X26 Y30=56, X26 X26=52, the threshold value=53.

In the hybrids between these races of different potencies of X and Y chromosomes there will appear some individuals in which the total potency of sex chromosomes is enough large to excite one group of genes, either female or male, in the autosomes introduced from one parent, so that the corresponding sexual characters will develop. For the sexual genes in another group of autosomes introduced from another parent it is at the threshold value or very near to it, so that some characters of both sexes may develop, and in such a case a female or male intersex will be produced. Sex reversal will be produced when the total potency of sex chromosomes passes over the threshold values in both parents to that side, male or female, which is opposite to the sexual constitution of the hybrid.

In the next table the statements of GOLDSCHMIDT on the different crosses are cited in the first column and the respective interpretation for each cross is described in the second. The cytoplasmic influence is denoted by the number 2; when the female parent is from the strong race 2 is added, and when it is from the weak or very weak race 2 is subtracted from the total sum of potencies in each zygote.

TABLE 11

Interpretations of Goldschmidt's basic crosses and crosses between strong and very weak races of Lymantria dispar.

J=JAPANESE STRONG RACE E=EUROPEAN WEAK RACE	j=AUTOSOME SET FROM JAPANESE RACE e=AUTOSOME SET FROM EUROPEAN RACE ⚥=INTERSEX. Rev=SEX REVERSAL
1. J♀×E♂=F_1 normal	X20 Y40×X25 X25=X20 X25, X25 Y40 45+2=47 65+2=67 ♂ ♀
2. E♀×J♂=F_1♂ normal, ♀ intersex	X25 Y37×X20 X20=X25 X20, X20 Y37 45−2=43 57−2=55 ♂ j♀, e⚥
3. (J×E)²=♀ normal, ♂ up to ½ of their number intersexual	X25 Y40×X20 X25= X20 Y40, X25 Y40, X20 X25, X25 X25 60+2=62 65+2=67 45+2=47 50+2=52 ♀ ♀ ♂ e♂, i⚥

J=JAPANESE STRONG RACE
E=EUROPEAN WEAK RACE

j=AUTOSOME SET FROM JAPANESE RACE
e=AUTOSOME SET FROM EUROPEAN RACE
⚥=INTERSEX. Rev=SEX REVERSAL

3a. (J×E)×(E×J)=exactly like No. 3	X25 Y40×X25 X20= X20 Y40, X25 Y40 X20 X25 X25 X25 60+2=62 65+2=67 45+2=47 50+2=52 ♀ ♀ ♂ e♂, j⚥
4. $(E\times J)^2$=♂ normal, ♀ ¼ normal and ¾ intersexuals	X20 Y37×X20 X25= X20 X20, X20, X25, X25 Y37, X20 Y37 40−2=38 45−2=43 62−2=60 57−2=55 ♂ ♂ ♀ j♀, e⚥
4a. (E×J)×(J×E)=exactly like No. 4	X20 Y37×X25 X20=same as No. 4
5. J×(J×E), J×(E×J): always normal	X20 Y40×X20 X25= X20 X20, X20 X25, X20 Y40, X25 Y40 40+2=42 45+2=47 60+2=62 65+2=67 ♂ ♂ ♀ ♀
6. E×(J×E), E×(E×J): exactly like F_2 No. 4, namely males normal, females ½ normal, ½ intersexual	X25 Y37×X20 X25= X20 X25, X25 X25, X25 Y37, X20 Y37 45−2=43 50−2=48 62−2=60 57−2=55 ♂ ♂ ♀ j♀, e⚥
7a. (J×E)×J=all normal	X25 Y40×X20 X20=X25 X20, X20 Y40 45+2=47 60+2=62 ♂ ♀
7b. (J×E)×E=♀ normal, ♂ all intersexual	X25 Y40×X25 X25=X25 Y40, X25 X25 65+2=67 50+2=52 ♀ e♂, j⚥
8a. (E×J)×J=all ♂ normal, ♀ intersexual	X20 Y37×X20 X20=X20 X20, X20 Y37 40−2=38 57−2=55 ♂ j♀, e⚥
8b. (E×J)×E=all normal	X20 Y37×X25 X25=X20 X25, X25 Y37 45−2=43 62−2=60 ♂ ♀
a. Very weak ♀×Strong ♂=only ♂	X26 Y30×X20 X20=X20 X26, X20 Y30 46−2=44 50−2=48 ♂ Rev♂
b. Strong ♀×Very weak ♂=normal	X20 Y40×X26 X26=X20 X26, X26 Y40 46+2=48 66+2=68 ♂ ♀
c. Very weak ♀×F_1 ♂ from b=3♂:1♀	X26 Y30, X20 X26= X20 X26, X20 Y30, X26 X26, X26 Y30 46−2=44 50−2=48 52−2=50 56−2=54 ♂ Rev ♂ ♂ ♀
d. (Strong ♀×Very weak ♂)²=2♀, 1♂, 1♀ by sex-rev. (hardly viable)	X26 Y40×X20 X26= X26 X20, X20 Y40, X26 X26, X26 Y40 46+2=48 60+2=62 52+2=54 66+2=68 ♂ ♀ Rev ♀ ♀
e. (Strong ♀×Very weak ♂)♀×Very weak ♂=only ♀	X26 Y40×X26 X26=X26 X26, X26 Y40 52+2=54 66+2=68 Rev ♀ ♀

The appearance of abnormal sex in *D. melanogaster* (BRIDGES 1922, 1925) may also be elucidated on the basis of our present supposition. The triploid intersex carries triploid autosomes and two X chromosomes (3A, 2X). The total of the potencies of two X chromosomes may be here considered to be equal or very near to the threshold value for three sets of autosomes, and consequently both sexual characters are revealed. The super-female (2A, 3X) and male (3A, X) are considered to be caused by extremely excessive and scanty quantities of the exciting factor respectively, of which the sterility is the result.

In bees and many other Hymenoptera the diploid egg develops into a female and the haploid into a male. These facts are intelligible when we assume a cytoplasmic influence upon the potencies of sex chromosomes. We suppose that the cytoplasm in Hymenoptera resists the exciting action of sex chromosomes and weakens it somewhat. In a diploid egg the sum of the potencies of two sex chromosomes, 2x, decreased by the quantity c, the cytoplasmic resistance, i.e. 2x-c, might still be greater than the threshold value for two sets of autosomes, and stimulate the female genes to their activity, while in the haploid form x-c is less than the threshold value for one set of autosomes, and the male genes only will be excited.

Thus the hypothesis of quantitative differences in the degree of sensitivity of the male and female sexual genes in autosomes and corresponding differences in the potencies of sex chromosomes in different sexes explains well many complex facts of sex differentiation, and I think that it is the general mode of sex differentiation.

Through this hypothesis the phylogenetic relationship among the hermaphrodite, rudimentary hermaphrodite (KOSSWIG 1931, WITSCHI 1929) and the gonochorist may be well cleared up. In the hermaphrodite the sensitivity of male and female genes to stimulating action is equal, and all of them are consequently excited to the same degree. In the rudimentary hermaphrodite, however, the sensitivity of sexual genes and the corresponding total potency of sex chromosomes is different in male and female sexes but this difference is small and the total potency of sex chromosomes in each sex draws very near to the threshold value. In such animals some slight influence of outer or inner conditions will induce the disturbance of sex differentiation, sex reversal or intersexuality being easily produced. In the gonochorist this difference is so great that normally any condition is unable to cause such sexual disturbances.

The three sexual types i.e., Protenor ♀XX♂XO, Drosophila ♀XX-♂XY and Abraxas type ♀XY♂XX show according to our hypothesis only the differences in respect to the accumulation of stimulating genes in two sex chromosomes. In the Protenor type all genes are contained in the X chromosome only and in the latter two types they are distributed

between two chromosomes X and Y. In the Drosophila type the X chromosome carries a greater number of genes than Y, and in the Abraxas type on the contrary the Y chromosome is greater in its potency than the X. Crossing over of the stimulating genes, if possible, might produce one type from another. The difficulty that opposite types of sex determination appear in very nearly related species, for example, the Drosophila type in Lebistes and the Abraxas type in Platypoecilus may thus be easily explained.

SUMMARY

Further breeding investigations on the abnormal males of *Aplocheilus latipes*, which were assumed in my former paper (1927) to be the products of non-disjunction of the sex chromosomes are described. These males produce in crosses with normal females offspring in which the number of females always exceeds that of males in varying ratios.

The sex ratios in these offspring vary according to the individualities of male and female parents. The different males from the same litter yield offspring of different sex ratios, and also the different females mated to the same male produce offspring of varying sex ratio.

Temperature influences the sex ratio which may differ in the offspring of the same parents produced early in the year or during mid-summer. Generally under a hotter climate the number of male offspring is greater than under a colder one.

By continued breeding of males selected out from the litters with high ratios of males the number of males increases until eventually some males are obtained whose male offspring far exceed the females in number. This fact denies the supposition of non-disjunction as accounting for the origin of abnormal males, so that it must be discarded and the alternative of sex reversal adopted.

The sex-reversed female of male genotype XY was found. It produced in crosses with normal males female and male offspring in the ratio of 1:3. One-third of its male offspring were males of the constitution YY, and these produced only male offspring.

For the interpretation of these results a new hypothesis of sex differentiation is proposed. The female and male primary sexual characters have all their own respective corresponding genes; these are distributed in the autosomes, and are set into activity by a certain amount of stimulating genes. The degree of sensitivity of female and male genes to the stimulating genes is various, and the female genes require a greater amount of stimulation than the male genes to become active. Sex differentiation is caused by differences in quantity of the stimulating genes. When the difference is great the female genes alone are activated and the action of male genes suppressed, while if it is small just the reverse takes place. Between these

two quantities is a threshold value, above or below which the female or male genes are stimulated to produce female or male respectively. The genes of stimulation in the gonochorist are located in the sex chromosomes, and the total stimulating capacity or potency is greater in female sex chromosomes than in male ones.

Sex reversal in our fish and the differences in the sex ratios among the offspring of sex-reversed males are explained by the fluctuation of the stimulating power or potency of the X chromosome.

Other abnormal facts of sex differentiation are explained on the basis of our present hypothesis.

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